Welcome to the 8th World Congress of Veterinary Dermatology. One of the primary purposes of the World Congress is the dissemination of information on current diagnostic and treatment modalities in the various fields within our discipline. The Continuing Education Program of the World Congress is focused on this goal.

The 8th World Congress is dedicated to the memory and achievements of our friend and colleague, Dr. Didier-Noël Carlotti, who was the inspiration and driving force behind holding this Congress in Bordeaux. Didier believed that continuing education in veterinary dermatology was extremely important and he shared his enthusiasm and knowledge of the discipline with veterinarians in many locations throughout the world. We miss Didier greatly but know that he would have been proud of our efforts to deliver this high-quality Continuing Education Program at the World Congress.

We also lost our friend and colleague, Dr. Peter Ihrke, since the last World Congress in Vancouver. Peter also understood the importance of continuing education in veterinary dermatology — he lectured and worked with veterinarians worldwide to spread current, practical information about the discipline. To recognize Peter and his passion for veterinary dermatology and continuing education, one of the continuing education streams is named in his honor.

The Proceedings are a culmination of many hours of thought and work by the authors, Program Committee and Publications Committee. We hope you find the information interesting and relevant. We are also thankful to the sponsors for their support of the World Congress, which made publication of these proceedings possible.

Sonya Bettenay
Catherine Outerbridge
Co-Chairs, Program Committee, WCVD8

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APPROACH TO THE DERMATOLOGY PATIENT

Claudia S. Nett-Mettler

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1. Introduction
   One of the main reasons that dermatologic patients can become frustrating for veterinarians is lack of time for a thorough dermatological work-up. In general, dermatologists devote at least one hour of their time for the first time appointment. In a busy small animal practice this may be difficult to achieve, however, most owners will understand that a skin exam takes additional time and therefore will be willing to come for a more thorough work-up at a later time if necessary. To be successful in veterinary dermatology, it is important to choose a systematic approach and devote enough time for a thorough history, physical and dermatological exam.

2. History
   History-taking is the first but nevertheless a very essential tool to access the dermatological patient. This can take up to 20-30 minutes of the consult time. On the other hand a thorough history can already lead to the diagnosis or at least narrow down the differential diagnosis lists.

A. Signalment
   The signalment decreases the differential diagnoses as well. Breed predispositions, age of onset and in a few diseases also the sex can help to include or exclude differential diagnoses.
   i. Age at onset
      Some dermatological conditions are age related: Young animals are predilected to infectious diseases (e.g. demodicosis, cheyletiellosis, lice infestations, dermatophytosis) as well as congenital diseases, in contrary, old animals are predilected to autoimmune diseases and tumors. Allergic dogs usually show the first symptoms of itch at the age of 6 months to 3 years often suffer from allergic diseases.
   ii. Breed
      Many canine dermatological diseases show strong breed predilections: Demodicosis in pugs, bulldogs and Westies, alopecia X in Pomeranians, zinc responsive dermatoses in Huskies, ichthyosis in Golden Retrievers, dermatomyositis in Shelties and Collies, nodular dermatofibrosis in German Shepherds, sebaceous adenititis in Akita Inus and Viszlas. In cats breeds are of less importance, however Devon rex cats commonly suffer from malassezia dermatitis. The breed can therefore help to develop and prioritize differential diagnosis lists.
   iii. Sex
      Only a few dermatoses are sex related, the most common is sex hormone alopecia in male dogs with testicle tumors.
B. Pruritus
Pruritus is an important complaint in many dermatological diseases. It is extremely beneficial for prioritizing the differential diagnoses to define whether pruritus was prior to lesion development or secondary. Pruritus prior to any lesion development is a hallmark of allergic dermatitis but can also be seen in animals with infectious diseases as for example sarcoptic mange, cheyletiellosis, flea infestation as well as cutaneous lymphoma. Pruritus secondary to lesions is unspecific and mainly due to secondary infections with bacteria or malassezia.

C. Seasonality
Seasonality of dermatological diseases is mainly due to seasonal atopic dermatitis or seasonal infectious diseases such as trombiculiasis and flea infestation.

D. In-contact animals and humans
If in-contact animals or humans are also suffering from skin lesions or pruritus, contagious infections such as sarcoptic mange, cheyletiellosis and dermatophytosis must be considered.

E. Travel history
Some infectious skin diseases (leishmaniasis, oomycetes and deep fungal infections (e.g. lagenidiosis, pythiosis, sporotrichosis), *Dirofilaria repens* and trombiculiasis are endemic to certain areas or countries. The travel history helps to include or exclude such infections from the differentials list.

F. Previous diagnostic tests and their results
Previous test results are often beneficial when writing formulating the differential list. However, these results need to be carefully interpreted based on concurrently applied drugs, time elapsed since the test was taken, as well as the time point when the test was performed (allergy testing during the winter months might miss a grass pollen allergy).

G. Responsiveness to previous therapies
Response or non-response to previously applied therapies is also useful to reduce the number of differential diagnoses. Improvement of skin lesions and pruritus under corticosteroids (and other immunomodulatory drugs) makes allergic dermatitis more likely; worsening of skin lesions (but not necessary of pruritus) under corticosteroids is typical in the presence of infectious diseases such as dermatophytosis, sarcoptic mange or demodicosis. Improvement under antibiotics implies a bacterial component of the disease and improvement under antiparasitic drugs implies an infestation with parasites such as fleas, mites or lice.

3. Physical findings
A. Physical exam
Several systemic diseases can present with dermatological symptoms with the skin
acting as a mirror for internal disease. Therefore a thorough physical examination should always precede the dermatological examination since in some diseases, dermatological symptoms occur prior to the systemic symptoms. The most common systemic diseases presenting with dermatological symptoms include: endocrinopathies (hypothyroidism, Cushing’s disease, sex hormone alopecia), infections (leishmaniasis), neoplasia (superficial necrolytic dermatitis, paraneoplastic alopecia (pancreatic carcinoma, bile duct carcinoma), exfoliative dermatitis (thymoma), systemic histiocytosis, autoimmune disease (systemic lupus erythematosus, uveodermatomatological syndrome))

B. The dermatological exam
After having collected all physical abnormalities, the skin and hair coat are examined. An adequate light source is essential and it is best to do this in a systemic manner including all visible mucous membranes, interdigital spaces, nails, paw pads. The skin is evaluated for its texture, elasticity, thickness, temperature and presence of skin lesions. The hair coat is evaluated for its texture and density and whether hairs can be epilated easily. An otoscopic examination should be part of each dermatological exam. Pinnae, ear canals and tympanic membrane are inspected and the quantity and quality of ear exudate recorded.

C. Skin lesions
During the exam, all lesions are carefully palpated, recorded and divided into primary and secondary lesions. After the exam, a differential list is made up with all the lesions recorded. Primary lesions are especially helpful for that list, whereas secondary lesions are mainly due to self-trauma and pruritus or remnants of primary lesions. Therefore a primary lesions is of more value in contributing to the differential diagnoses than secondary lesions
i. Primary lesions
   Primary lesions include macules/patches, papules/plaques, pustules, bullae, vesicles, wheals, nodules, cysts
ii. Lesions that may be primary or secondary
   Alopecia, scales, crusts, follicular casts, comedones, pigmentary abnormalities
iii. Secondary lesions
   Epidermal collarettes, scars, excoriations, erosions, ulcers, fissures, lichenification, callus formation

D. Lesion distribution
The lesion distribution or the areas affected with pruritus give important clues towards the diagnosis and help to prioritize the list of differentials. Lesions can be localized, multifocal or generalized, symmetric or asymmetric. Symmetric lesions usually reflect an internal cause (e.g., endocrine or metabolic disease) whereas asymmetric lesions are typical of infectious causes or neoplasia.

Many dermatological conditions show a typical lesion distribution pattern and some diseases typically preferably affect specific areas of the body. Such regional
dermatoses included dermatoses affecting the head, ear, nose, eyelid, nasal planum, lip, mucosae and mucocutaneous junctions, further chin, neck, anus, paws and claws. Examples include symmetric lupoid onychodystrophy (claws), discoid lupus erythematosus (nose, planum nasale), nasodigital hyperkeratosis (planum nasale, paws), lip fold intertrigo (lips), indolent ulcer (lip, oral cavity), perianal fistulae (anus). Tables with lists of differential diagnoses provided in standard dermatology textbooks are helpful when encountering regional dermatoses.

4. Problem list, differential diagnosis and minimal data base
Skin diseases can have a variety of different causes and since the skin only has a limited number of responses to insults, skin diseases often look alike and frequently it is necessary to run a number of diagnostic tests to achieve a definitive diagnosis.

After having collected all data of the history, physical and dermatological exam, the problem list is formulated. Based on the problem list, a prioritized list of possible diagnoses is developed and diagnostic tests are chosen and performed to minimize this list in order to find the definitive diagnosis. The minimal data basis includes those diagnostic tests, which are helpful to define the definitive diagnosis in an easy, quick, and inexpensive way. For example, in the presence of papules and pustules the minimal data basis would include pustule cytology, skin scrapings, trichogram and wood lamp exam. With those few, easy to perform and inexpensive tests, infectious diseases causing papules and pustules would be diagnosed. If the diagnosis cannot not be established with the minimal diagnostic database, further necessary diagnostic steps or therapeutic trials should be discussed with the owner.

5. Summary
To successfully access the dermatological patient, a detailed history and a thorough physical and dermatological exam are the first essential steps. Differentiating primary from secondary lesions and choosing diagnostic tests based on a detailed differentials list are further important steps to successfully make a final diagnosis. However, it is essential to always address the owner’s main complaint in order to achieve client satisfaction.

Selected References
THE DIAGNOSTIC APPROACH TO PRURITUS

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Why diagnose – let’s just treat!
Although it may be tempting to just employ a drug marketed for the treatment of pruritus, there are two main reasons why this is very bad practice: (i) this will not cure the disease, and (ii) there is no such thing as a drug without side-effects. So the goal must be to define the nature and cause of the disease as precisely as possible and then to apply treatment tailored to the condition.

How does the dog manifest pruritus?
There seems to be much variation in both the thresholds of different dogs and also in the behaviour shown. Recent studies have shown that IL-31 is a key cytokine in the pathway leading to pruritus in canine atopic dermatitis (CAD) (Gonzales et al., 2013). But dogs reacted differently to injection of the recombinant cytokine, with some scratching, some licking and others head-shaking. Some dogs roll on their backs or rub against the furniture. In the chronic pruritic dog one will expect to see erythema, excoriations and hair loss which in the long-standing case leads to hyperkeratosis, crusting and scaling and ultimately hyperpigmentation.

Similarly to dogs that have varying thresholds, owners have differing perceptions of the severity of itching. It is thus useful to ask the owner to use a numerical (1-10) or visual analogue scale, which has at one end no itch, and the other the worst imaginable itch where the dog wakes up at night scratching, and ultimately stops to scratch when walking or eating. Also important is the distribution of the pruritus – is it localized, if so where, or generalized?

How does the cat manifest pruritus?
Cats tend to respond to pruritic stimuli predominantly by licking and rubbing and to a lesser extent scratching. But cats that do scratch tend to induce severe excoriations. The cat has rather typical reaction patterns of self-induced alopecia (either symmetrical or largely ventral), head and neck pruritus, miliary dermatitis, and the eosinophilic granuloma complex (eosinophilic plaques, indolent ulcers and linear (collagenolytic) granulomas) associated with pruritic diseases.

Pointers from the history
The importance of obtaining an accurate history has been emphasized in the previous presentation, and will not be repeated. It may enable you to raise your index of suspicion for one or more diseases – for example is there a history of possible contagion – such as visiting the veterinarian’s office, going to a dog show? Or does the dog walk in a rural area with possible exposure to foxes (scabies) or rabbits (cheyletiella)? Is the condition seasonal or perennial (obviously difficult to ascertain on the first occurrence!). Are the owners affected (pointers to scabies or cheyletiella)?
The physical examination
Thoroughness is essential! Is there evidence of self-excoriation, and what is its distribution? What is the distribution of any hair loss, and can its extent be explained by the severity of the pruritus, or might there be some concomitant condition contributing to the hair loss? Are there primary lesions, and what is their nature and distribution? What secondary changes are evident? Is there evidence of fleas and/or flea dirt? Is there any sign of lice or the lice nits?

Is there evidence of a pyoderma? Especially if superficial, this can be very pruritic. If the condition improves with antibacterial therapy, but the pruritus remains, this is suggestive of an underlying allergic disease.

Cytological examination and skin scrapings

Impression smear cytology. A clean microscope slide is pressed on the lesional area. If there are pustules present they can be ruptured with a 26g needle prior to application of the slide. The slide is generally heat fixed and then stained with haematoxylin and eosin.

Cellulose acetate tape strip preparations. These should be done on areas suspicious of *Malassezia* or bacterial overgrowth – both of which can be very pruritic. After trimming any hair away with scissors, the tape is firmly pressed down and removed. The tape is NOT fixed, but stained with the haematoxylin and eosin stains, placed on a microscope slide and examined. Surface dwelling parasites such as *Cheyletiella* and *Trombicula* can be identified by these means.

Skin scrapings. Both superficial and deep scrapings are taken sequentially from the same areas – superficial looking for *Cheyletiella* and the male *Sarcoptes* mite, (or *Notoedres* in cats) and deep looking for demodex and the female *Sarcoptes*.

Rule-outs for the dog
Firstly, the old saying that “common diseases occur commonly” should be remembered when approaching the pruritic patient. So this presentation will not discuss the rarer causes of pruritus – only those likely to be seen regularly in first opinion practice. Secondly, remember that CAD is a diagnosis by exclusion, and so it is important to rule out other diseases before considering this diagnosis.

Scabies. This is still a fairly common disease, despite the routine use of parasiticidal agents that are scabicidal. Important pointers are:

i. Are other dogs in the household infected? Remember that it may take up to several weeks for transmission to occur between pets, depending on the length of hair, which can be protective, and the closeness of the pets.

ii. Are the owners affected? They are in some 50% of cases and pruritus can commence within 12 hours of contact with the affected dog.

iii. Is there a history of possible contagion? Whence could the animal have acquired the infection?

iv. Is it a ventral disease (typically including the ear margins)? Scabies almost never goes dorsally.
v. Is there a positive pinnal-pedal reflex? Rubbing the edge of the pinna induces a scratching reflex. This was positive in 82% of cases as compared with only 6.2% of other pruritic skin diseases in a report (Mueller et al, 2001).

vi. Superficial and deep skin scrapings are likely to be positive in only some 50% of cases.

vii. Is the pruritus corticosteroid responsive? Typically, cases of scabies respond only partially (around 50%) to corticosteroids, as opposed to 100% with most allergic diseases – but it is not suggested to try this approach diagnostically!

viii. Serology for scabies-specific IgG. This is marketed in Europe with varying reports of its specificity and sensitivity.

ix. If in doubt, use trial therapy.

Cheyletiella. Some of the pointers are similar to those for scabies.

i. Are the owners affected?

ii. Are other dogs in the household affected?

iii. Where could the animal have acquired the infection, e.g. walks in the countryside infested with rabbits, veterinarian’s office, other forms of close contact with dogs?

iv. Use tape strips after carefully trimming the hair, or superficial skin scrapings, or examine hair shafts for eggs.

v. In most cases a positive diagnosis can be made, but if the history and clinical signs point to cheyletiellosis, trial therapy should be employed in the absence of a definitive diagnosis.

Trombicula (harvest mites or berry bugs). These have a limited season in some parts from August through September with a more extended season in others. The mites are just visible to the naked eye as little orange dots with the sites of predilection between the toes and on the ear flaps.

Lice. Biting (Mallophagia) or sucking lice (Anaplura) now have a fairly restricted geographic distribution and are readily visualized as are the shiny nits.

Demodex. This is not ordinarily pruritic unless there is a secondary pyoderma. Deep skin scrapings or examination of purulent material from the pyoderma will readily enable exclusion of this condition.

Flea allergy dermatitis (FAD). This has a typical distribution in dogs favouring the lower back and posterior thighs. The mere presence of fleas does not justify a diagnosis as in the absence of an allergic response, flea infestation itself can be non-pruritic or only mildly pruritic. The diagnosis requires:

i. The presence of fleas and/or flea dirt – using coat brushings if necessary.

ii. Compatible distribution.

iii. Evidence of hypersensitivity which is demonstrable by intradermal testing - looking for an immediate reaction (IgE) or a delayed (cell-mediated) reaction at 24-48 hrs. If either is positive, the diagnosis of hypersensitivity is made. Alternatively serology can
be employed, but only some 80% of dogs and cats that are clinically allergic will be positive with the others suffering only from delayed hypersensitivity.

**Ticks and tick bite hypersensitivity.** In areas in which ticks are prevalent, adult ticks can cause severe granulomatous reactions, and the small, larval stage can be a cause of severe pruritus.

**Malassezia dermatitis.** This has a rather typical appearance and affected areas are usually greasy and hyperkeratotic. There are varying reports regarding the numbers of organisms required to make a diagnosis ranging from > 2 per high power field to >10 in 15 randomly selected oil immersion fields. But caution is required. In some suspected cases anti-fungal treatment will eliminate the organisms but have no effect on the level of pruritus. In others, sometimes with smaller numbers, antifungal treatment has a dramatic effect on the pruritus. Two factors are important:

i. Remember that Malassezia overgrowth is usually secondary to an underlying disease and,

ii. Hypersensitivity to the organism may be responsible for the clinical signs. This is the rationale for undertaking serology for Malassezia-specific IgE.

**Bacterial overgrowth and pyoderma.** Bacterial overgrowth may occur on its own or concomitantly with *Malassezia* dermatitis. Tape strips or impression smears will show abundant cocci and sometimes there is neutrophil extravasation with active phagocytosis. Lesions are generally erythematous and greasy.

Superficial pyoderma - impetigo or folliculitis – is readily diagnosed by the presence of pustules – however in the latter, the pustular phase is often short and most observable lesions are papules, often with spreading crusting lesions and the presence of epidermal collarettes. Superficial pyodermas are variably pruritic. Again, as in the case of *Malassezia*, remember that:

i. Pyoderma usually results from an underlying disease – most commonly CAD, and

ii. The associated pruritus may be the result of the underlying disease or, possibly, hypersensitivity to the organism.

**Rule-outs for the cat**

Parasitic diseases are again the most important rule-outs. *Feline scabies.* Caused by *Notoedres cati*, this highly pruritic disease has a characteristic distribution involving the head and neck. It occurs in clusters with a limited geographic distribution and is absent from many parts of the world. The mite can also cause a pruritic rash in man. Mites are readily demonstrated in skin scrapings.

*Cheyletiella* is not uncommon in cats. It can cause varying degrees of pruritus and an asymptomatic carrier state can also exist. Diagnosis is as for the dog.

*Demodex gatoi*, which is a pruritic form of demodex in the cat can be ruled by superficial skin scrapings.
**Trombicula.** This may cause severe pruritus in cats and is most commonly found interdigitally or in the folds of the ear flaps.

**Otodectes.** The ear mite can leave the ear canal and infest the head and neck region. They are just visible with an otoscope in the ear canal and may be recovered in skin scrapings. There is a well-documented hypersensitivity reaction in cats and pruritus can be quite severe.

**Lice** are occasionally seen as a cause of pruritus and are readily visualized.

**Flea allergy dermatitis** is undoubtedly the most common feline skin disease worldwide and can be a cause of any of the reaction patterns. Probably the most common presentation is symmetrical hair loss or “bald belly syndrome” +/- military dermatitis but the distribution can be varied often favouring the head and neck. As cats are such efficient groomers, it is often hard to find evidence of infestation. Hypersensitivity is demonstrable by skin testing or serology, but evidence of hypersensitivity on its own does not justify a diagnosis. It is wise to rule out the involvement of fleas by trial therapy.

**Psychogenic alopecia.** This excessive grooming tendency is most commonly reported in Siamese and Burmese cats but may be overdiagnosed. It is discussed further under the allergic diseases below.

OK – we have ruled out all the pruritic diseases noted above. What is next for the pruritic dog?

We now have to consider atopic dermatitis (CAD) or an adverse food reaction (AFR). But some fundamentals must be discussed before we proceed.

**CAD and AFRs – are they the same disease?**

There is much controversy on this topic. CAD is classically associated with environmental allergens that gain access percutaneously. They were always considered separately from AFRs, whilst acknowledging that they may occur concomitantly in the same patient. In man, they tend to be considered as one disease and many patients are treated symptomatically, without a separate investigation of the role of aeroallergens and foods. Arguably, the traditional veterinary approach which is to assess the role of foods as a starting point before investigating aeroallergen involvement, may lead to a better therapeutic outcome.

The terms Food Induced Allergic Dermatitis (FIAD), and Atopic Dermatitis sensu stricto (CADss) to describe classical CAD associated with aeroallergens were introduced by Picco et al in 2008. The above authors also defined some differences between FIAD and CADss which included:

i. FIAD tends to start in the younger dog with 48% showing clinical signs at < 1yr as opposed to 16% of dogs with CADss.

ii. There is a higher incidence of concomitant gastrointestinal signs in FIAD.

iii. There is a higher incidence of Malassezia involvement in FIAD.

iv. There is no seasonality in FIAD whereas 35% of dogs with CADss show a seasonal pattern.
v. In addition to the breeds predisposed to CADs, the German shepherd, pug and Rhodesian ridgeback are predisposed to FIAD.

Some have extended this to use the term Food Induced Atopic Dermatitis for an AFR – whilst still recognizing that AFRs on occasions have quite different presentations. Ultimately, however, it is really immaterial what terminology is applied so long as one considers the role of food and aeroallergens separately. For the remainder of this discussion the original terms of CAD and AFR will be used.

Diagnosing an AFR in dogs

*What is the pathogenesis?* There is evidence that some cases are IgE mediated, but it is also apparent that cell-mediated (delayed) hypersensitivity is involved. Patch testing and the specialized laboratory assay lymphocyte blastogenesis have very good sensitivity and specificity. Unfortunately they are either cumbersome to perform (the former) or not readily available (the latter). There is some evidence that enzyme-linked immunosorbent assays for food allergen-specific IgE or IgG may be helpful in selecting a limited allergen diet as they have a high negative predictive value (>80%). However they cannot be used to implicate the allergen.

*What is the diagnostic “Gold Standard.”* It is universally agreed that the only way to make a diagnosis is by observing a reduction or cessation of the degree of pruritus on feeding a hypoallergenic diet exclusively, followed by a relapse upon reinstituting the original diet or components of it. The diet should be fed for up to 8 weeks or until a response is seen. It is perfectly acceptable to administer anti-pruritic doses of corticosteroids for the first 2 weeks to give some relief, then withdrawing to ascertain if the diet is proving effective. Upon challenge, most dogs relapse within 3 days but some may take up to 2 weeks.

*What diet should be used?* The following are available:

i. Limited antigen commercial diets. These have not proven very useful with only some 40-60% of cases responding – possibly due to contamination by other proteins.

ii. Hydrolyzed diets. These should provide the optimal approach but at the time of writing none have been shown to be 100% reliable with some 80-90% of cases responding. There has been some work that suggests that some such diets may contain higher molecular weight contaminants.

iii. Home prepared single source protein diets. Arguably, this is the optimal approach for diagnosis, then assessing various commercial diets for maintenance. The protein source is chosen either by a careful dietary history and avoiding any possibly cross-reacting diets (for example, beef, lamb and dairy products cross-react) or on the basis of negative serology. Potato is a suitable carbohydrate source.

*What results can I expect?* There may be (i) a total, (ii) partial or (iii) no response. If it is (i), then the diagnosis is made. If it is (ii) we have a partial diagnosis and we should pursue the possibility of CAD for the other part. If it is (iii), we again pursue CAD.
Diagnosing an AFR in cats

AFRs can result in any of the reaction patterns noted for the cat and the same diagnostic approach is followed as for the dog taking care to ensure adequate taurine content of any home-prepared diet. However, in cats there is the special challenge of psychogenic alopecia which is also largely a diagnosis by exclusion – as is the case with AD. It affects predominantly Burmese and Siamese but is less common than is generally believed. In a recent study in Canada, 20 dogs with symmetrical alopecia resulting from excessive grooming were referred with this diagnosis but in only two cases was psychogenic alopecia the final complete diagnosis (Table 1)

Table 1. Final diagnosis in 20 cats referred with a diagnosis of psychogenic alopecia (Waisglass et al., 2006)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adverse food reaction + atopic dermatitis</td>
<td>5</td>
</tr>
<tr>
<td>Adverse food reaction</td>
<td>5</td>
</tr>
<tr>
<td>Undiagnosed</td>
<td>2</td>
</tr>
<tr>
<td>Adverse food reaction + atopic dermatitis + flea allergy</td>
<td>1</td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>1</td>
</tr>
<tr>
<td>Undiagnosed parasitic</td>
<td>1</td>
</tr>
<tr>
<td>Undiagnosed parasitic + bacterial</td>
<td>1</td>
</tr>
<tr>
<td>Atopic dermatitis + psychogenic</td>
<td>1</td>
</tr>
<tr>
<td>Atopic dermatitis + adverse food reaction + psychogenic</td>
<td>1</td>
</tr>
<tr>
<td>Psychogenic alone</td>
<td>2</td>
</tr>
</tbody>
</table>

The final diagnosis by exclusion – canine atopic dermatitis (CAD)

If we have ruled out all of the conditions noted above or if there is only a partial response to a hypoallergenic diet, then a diagnosis CAD should be considered. This is defined as:

“A genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features with IgE antibodies most commonly directed against environmental allergens” (Halliwell et al, 2006)

All of these features have been evaluated for utility as diagnostic criteria on a number of occasions over the years with those proposed by Favrot and colleagues (2010) the most widely accepted. Nine criteria thought to be typical were evaluated in two sets and the specificities and sensitivities defined. These are:

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Set 1 or 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset &lt; 3yrs</td>
<td>Both</td>
</tr>
<tr>
<td>Mostly indoor</td>
<td>Both</td>
</tr>
<tr>
<td>Corticosteroid responsive pruritus</td>
<td>Both</td>
</tr>
<tr>
<td>Chronic or recurrent yeast infections</td>
<td>Both</td>
</tr>
<tr>
<td>Affected front feet</td>
<td>Both</td>
</tr>
<tr>
<td>Affected ear pinnae</td>
<td>Both</td>
</tr>
<tr>
<td>Non-affected ear margins</td>
<td>Both</td>
</tr>
<tr>
<td>Non-affected dorso-lumbar area</td>
<td>Both</td>
</tr>
<tr>
<td>Pruritus sine material at onset (without an underlying dermatosis)</td>
<td>Set 2</td>
</tr>
</tbody>
</table>
Obviously, not every criterion has to be fulfilled in every case. For example, dogs housed outdoors can certainly become atopic. And, of course, there will likely be an affected dorso-lumbar area if there is concomitant flea allergy dermatitis. But the criteria do provide guidance when making the final diagnosis.

What about atopic dermatitis in cats?
The condition is not as well defined in cats. Cases may be seen with any one or combination of the reaction patterns noted above. Also, concomitant respiratory signs may occur in some cases.

Should I perform an allergy test?
Intradermal tests (IDTs) or serology for allergen-specific IgE is undertaken in both dogs and cats for two reasons:
(i) To aid in the selection of allergens for immunotherapy. This is still regarded as the preferred treatment by many clinicians and,
(ii) To distinguish between CAD and Atopic-like Dermatitis (ALD). In the latter, there are characteristic clinical features of CAD, but no evidence of allergen-specific IgE. This should be identified as it may represent a different subset and respond differently to medical treatments. But, it must be remembered that the presence of allergen-specific IgE does not, of itself, justify a diagnosis, as positive reactions are often found in normal dogs.

And finally
The diagnosis has been made and the various approaches to treatment can be explored. But remember that there is no drug marketed just for the treatment of pruritus.

Selected References
Figure 1. Flowchart for the Workup of the Pruritic Dog

- Pointers from the history
  - Store for later use
  - Helpful but not diagnostic
  - Cytology and skin scrapings
- The physical examination
- Assess for parasites – Coat brushings
  - Negative
  - Positive
- Assess for pyoderma
  - Positive
  - Negative
  - Treat
    - Recurs
    - Cured
- Assess for AFR
  - Partial response
  - No response
  - Complete response
    - Diagnosis of AFR
    - Indications positive but IgE for environmental negative
      - Diagnosis of atopic-like dermatitis
    - Indications positive including IgE for environmental
      - Diagnosis of CAD +/- AFR
INTERPRETATION OF IN-HOUSE CYTOLOGY, TRICHOGRAMS AND DERMATOPHYTE CULTURES

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1. Introduction
In addition to skin scrapings for parasites, all cases with hair loss, scaling, crusting, papules, pustules or lichenification should be screened for infectious organisms with skin cytology. These quick and easy in-house tests will not only allow for the accurate diagnosis of the dermatitis and guide appropriate therapy, but are also revenue generators. Additionally, with the emerging problem of methicillin-resistant bacterial skin infections, cytology to monitor response to antimicrobial therapy is important and can guide decisions about culture submission. In addition to cytology of dermatologic lesions, evaluation of hairsafts and roots via trichography can add important information, and fine needle aspirate of skin nodules can screen for likely benign masses which may just need to be monitored vs. neoplastic tumors which should be biopsied or removed. Lastly, dermatophyte cultures are important tests for a zoonotic disease, and it is essential to know how to perform and interpret dermatophyte cultures correctly.

2. Skin cytology
Skin cytology can be used to obtain information on bacterial or Malassezia infections, as well as, to characterize inflammatory infiltrate. Samples are applied to a microscope slide and stained with a Romanowsky stain (Diff Quik), then observed under 40-100X. Neutrophilic or pyogranulomatous inflammation can be supportive of an infectious or inflammatory process, an eosinophilic infiltrate is supportive of a hypersensitivity dermatitis, and acantholytic cells can suggest pemphigus complex (but can also be seen with chronic bacterial or Trichophyton infection) and support the need for biopsy and histologic diagnosis. In some dermatophytosis cases, fungal arthroconidia or hyphae may be found on surface skin cytology. It is important to have a good microscope in order to perform accurate cytology, and fortunately microscope costs have decreased in the past 20 years; durable, easy to use and affordable microscopes are now available such as the Swift M10 Series Biological Lab Microscope. Make sure to train veterinary technicians in the correct ways to handle, clean and maintain microscopes; there are many online training resources, including http://micro.magnet.fsu.edu/primer/anatomy/cleaning.html.

A. Methods to obtain samples for skin cytology
   i)  If a pustule is present, it can be ruptured with a needle and the contents smeared onto a slide.
   ii) If a moist or greasy lesion is present, it can be sampled by firmly pressing a microscope slide on the surface of the lesion.
   iii) For dry scaling or diffuse crusting lesions, use of a dulled scalpel blade without mineral oil can be helpful to collect surface debris which is then smeared like a spatula onto the microscope slide. If larger crusts are present,
use the blade or microscope slide edge to raise the edge of the crust and then obtain an impression smear of the exudate or debris under the crust.

iv) For interdigital lesions, samples can be obtained via direct impression of the interdigital web onto a slide, cotton-tip swab of interdigital debris which is then rolled onto a slide, or by acetate tape impression (see below). In cases of paronychia, nailbed debris can be collected with a dull blade or the wooden end of a cotton swab, then smeared onto a microscope slide.

v) Acetate tape impressions can be used to sample dry, lichenified and interdigital areas. A piece of clear (not frosted) acetate tape is firmly pressed to the lesion, then applied onto a microscope slide over a few drops of blue Diff Quik or Lactophenol Blue stain and observed under 40-100X.

B. Beware of bacteria mimics
   i) Melanin granules can be round to oblong, and usually have a greenish brown, refractile appearance.
   ii) Keratohyalin granules are pink to purple irregular blobs which may be found within epithelial cells from the granular layer of the epidermis; they contain profilaggrin and keratin filaments.
   iii) Stain precipitate is amorphous, often granular debris.

3. Cytology of skin masses
   Skin masses can be due to tumors (benign or malignant), granulomas (infectious or immune mediated), abscesses, or cysts. In-house examination of fine needle aspirates (FNA) of skin masses is an important first step to determine further diagnostics and treatment. It is important to remember that some tumors exfoliate poorly and that a needle aspirate can miss certain tumors. Biopsy and/or excision would be indicated for masses in which in-house cytology is not diagnostic, or if a skin mass changes/enlarges.

A. How to obtain FNA samples
   i) Use a 22g needle and a 3 to 6cc syringe.
   ii) Insert needle into lesion, aspirate, redirect and aspirate again (if lesion is vascular, then only insert needle once then withdraw to avoid blood dilution of sample).
   iii) Take needle off syringe, draw air into syringe, replace needle and expel needle contents onto slide.
   iv) Use a second slide for gentle squash prep to form monolayer of cells.
   v) Stain with Romanowsky stain (Diff Quik), observe under 10X to find diagnostic area then observe under 40-100X.

B. Common skin masses which can often be identified in-house
   i) Follicular cyst/benign follicular tumor: usually amorphous keratinized debris +/- melanin granules.
   ii) Lipoma: often dissolve when stained, but residual lipocytes or non-staining lipid may be found.
   iii) Melanomas.
v) Other tumors: Large bizarre epithelial cells can be seen with squamous cell carcinoma, and is an indication for biopsy/excision.

C. Decision making via in-house cytology
i) Potential “wait and see” nodules: Follicular cysts, lipoma, histiocytoma.
ii) We need biopsy +/- staging lesions: mast cell tumor, lymphoma, plasmacytoma, melanoma.
iii) We need biopsy/special stains/tissue cultures: Pyogranulomatous inflammation.
iv) We need biopsy: everything else, including non-diagnostic FNA samples.

4. Trichograms
Trichograms can be helpful in cases of localized or generalized alopecia in order to evaluate anagen/telogen ratio of hair roots, investigate barbering potential, evaluate hair shaft integrity/damage, evaluate hair shaft pigment distribution in suspect cases of color dilution alopecia, and to screen for potential hair shaft infection in suspected dermatophytosis cases. Hairplucks for trichography can also be helpful to identify demodex mites in hard-to-scrape areas such as the lips and paws.

A. How to obtain and evaluate trichograms:
i) Gently pluck hairs with fingers or rubber tipped forceps to avoid iatrogenic breakage.
ii) In suspect M. canis cases, Woods lamp fluorescence can identify appropriate hairs for trichography.
iii) Place hairs in mineral oil and apply coverslip.
iv) Observe under 10-40X to assess root and hair shaft morphology.
v) Most animals will have a mixture of telogen (resting) and anagen (growing) hair roots.
vi) All telogen roots can suggest possible endocrinopathy or telogen defluxion (though keep in mind that Nordic breeds normally have telogen dominated hair cycles). Numerous anagen roots decreases suspicion for endocrinopathy.
vii) Color dilution causes irregular clumps of melanin within the hair shafts.
viii) Dermatophyte infected hairs show invasion of hair surface by fungal arthroconidia and hyphae, resulting in a pale, irregular appearance to the hair shaft.

5. Dermatophyte cultures
Dermatophyte culture is indicated in cases in which dermatophytosis is suspected clinically based on history, physical examination and/or involvement of other pets or humans in the home, and when no obvious bacterial, yeast or parasitic causes for hair loss can be identified.

A. Obtaining samples for culture: Reduce false negative cultures by using both the hair pluck technique combined with the toothbrush technique—brush a new toothbrush on
and around the suspect area, and then gently impress the bristles into the culture media. This is also the preferred way to culture possible asymptomatic dermatophyte carriers.

B. **Performing the culture:** Use rectangular or round culture plates rather than the harder to use screwtop tubes. Incubate fungal cultures at room temperature, and increase the humidity in the culture area (to reduce culture plate dessication) by placing culture plates in clear plastic bags or a partially covered plastic container which also contains a little dish of water. Monitor the cultures daily for 21 days — *Trichophyton* species tend to grow more slowly, as do cultures from animals undergoing antifungal treatment. Dermatophyte test medium (DTM) contains Sabouraud’s dextrose agar with cycloheximide, gentamycin and chlorotetracycline as antifungal and antibacterial agents to retard growth of contaminant organisms. Additionally the pH indicator phenol red is added. Dermatophytes preferentially metabolize protein in the culture medium, causing alkaline metabolites and turning the yellow fungal culture medium to a red color at exactly the same time as the dermatophyte colony appears. Most other fungi initially utilize carbohydrates with resultant acidic metabolites; these saprophytic fungi can eventually consume protein and cause media color change, but this happens several days after fungal growth occurs.

C. **Interpreting the culture:** Macroscopic fungal colony morphology is an important first step in determining if a dermatophyte is present. *Microsporum* and *Trichophyton* species, the most important dermatophytes in dogs and cats, appear as white, light yellow, tan, or buff-colored, cottony to powdery appearing colonies. Dermatophytes are never black, green, or grey. Additionally, in positive dermatophyte cultures, consideration of macroscopic colony numbers gives the clinician information on severity of infection and (in animals undergoing antifungal treatment) response to therapy. Microscopic evaluation of suspect fungal growth is also important, since some environmental fungi can mimic dermatophytes in gross colony morphology and ability to turn the media red, and since some strains of *M. canis* may not produce media color change. Gloves should be worn to avoid transmission of dermatophyte spores to the hands. A small piece of clear acetate tape is gently touched to the surface of the fungal colony and then the tape is applied to a glass slide over a drop of blue stain (such as methylene blue, lactophenol cotton blue, or the blue Diff-Quik solution (basophilic thiazine dye). The slide is examined under 10-40X for the characteristic dermatophyte macroconidia. In early cultures, only fungal hyphae with no macroconidia may be seen (especially in cases of *Trichophyton*), and these cultures should be incubated longer to allow for spore development. *Microsporum canis* has large spindle-shaped, thick-walled spores with a terminal knob and 6 or more internal cells. *Microsporum gypseum* produces large spindle-shaped spores with thin walls, no terminal knob, and 6 or less internal cells. *Trichophyton mentagrophytes* produces long cigar-shaped macroconidia with thin walls; spiral-shaped hyphae and numerous small grape-like clusters of microconidia are also characteristic of *Trichophyton*. In cases in which the fungal species cannot be easily identified in the clinic, then the
dermatophyte culture should be submitted to a veterinary reference laboratory for fungal identification.

Summary
Skin diseases are one of the most common reasons that pets are brought into veterinary hospitals. With practice, cytological analysis, trichograms and dermatophyte culture interpretation are easy to do, and will maximize our ability to appropriately diagnose and treat these patients.
Selected diagnostic tests in the pruritic patient

1. Serum sarcoptes tests
   A. **Pros:** In 2001 two independent studies concluded that the newly developed scabies serum test in dogs was “useful in the diagnosis of canine scabies”. This test is rapid and has been helpful to owners who may be otherwise reluctant to use an ectoparasitic agent. It is however “useful” but not “100% infallible” - in neither study was the test 100% reliable – both false positives and false negatives were reported.
   
   B. **Cons:** In dogs, the first of these studies, 16 / 19 dogs positively identified by skin scraping was positive on serology and 4/38 control dogs showed a positive result. In the second study, 2/12 infested dogs were negative on serology and 2/13 healthy control dogs were positive. In humans, Larry Arlian had already demonstrated in the early 1990’s, that antigens of the parasitic mite *Sarcoptes scabiei* cross-react with antigens of the house dust mite *Dermatophagoides pteronyssinus*. Despite more than three decades of research a recent publication by this author – a giant in the field of scabies research - still concluded that “co-sensitization or cross-reactivity between antigens from scabies and house dust mites confounds developing a blood test for scabies.” 73.6% of the (human) scabies patients were identified to have serum IgM that recognized scabies proteins, and all except two of them also had IgM that recognized all of the three species of dust mites.
   
   C. **What do I do?:** I use an ectoparasitic diagnostic therapy in all cases of pruritus. In recent times, there have been anecdotal reports of resistance of scabies mites to the standard diagnostic therapies (milbemycin, selamectin and moxidectin) and thank goodness, the newly released isoxazalines, insecticide and antiparasitic agents (afoxolaner, fluralaner, sarolaner) have been shown to be efficacious and mite resistance is unlikely.

2. Serum IgE testing and the diagnosis of atopy
   Allergy testing and the diagnosis of atopy are discussed in detail in other talks and workshops during this congress and will not be covered in detail here.
   
   A. **Pros:** Serum IgE and intradermal allergy testing (IDAT) for environmental allergens may be used - in rare cases - to avoid an allergen. Heavy pollens such as privet (*Ligustrum* spp.) may truly be avoided by choosing to walk a different route during pollination season, grass pollens are not so easily avoided. A serum IgE allergy test (environmental) is most frequently used to formulate an allergen specific therapy.
The negative results of a food serum IgE test can be used with approximately 80% accuracy to choose an elimination diet protein source.

B. Cons: False positives and false negatives occur. The interpretation requires correlation of clinical relevance of a positive test result with the serum IgE value. This requires a detailed knowledge of both the animal and allergen. For example seasonality – does the plant pollination time match the clinical history? A positive serum IgG should be interpreted to reflect exposure to an allergen, not a clinically relevant hypersensitivity. The negative results of a food serum IgE test can ONLY be used with approximately 80% accuracy to choose an elimination diet protein source so 1/5 times this is wrong.

C. What do I do?: I use serum IgE tests (or IDAT) to formulate allergen specific therapy. I do not recommend serum allergy tests to my clients as part of my diagnostic work-up. Environmental allergy is a diagnosis of exclusion – food allergy and scabies must be ruled out prior to initiating allergen specific therapy. I do not use serum IgE tests for food allergens, the results are misinterpreted by most owners. The use of a negative value to select a protein for the elimination diet is not 100% reliable and we often get just one shot at the diet. A food hypersensitivity is best diagnosed using a carefully selected elimination diet with close monitoring and extensive follow-up although Mueller’s group has established that a food allergen patch test may be helpful to help choose which protein to feed during this diet.\(^5\)

**Selected diagnostic tests in the alopecic patient**

Focal, multifocal, symmetric, inflammatory and non-inflammatory hypotrichosis and alopecia are often associated with inflammatory / infectious causes.

1. Approach to the alopecic patient
   - **Step 1:** look at the history and the “whole animal”. Look for clues for: Primary follicular diseases, systemic diseases, causes of infectious folliculitis, pruritus? There are many clinical presentations which may be associated with follicular infections and the 3 most common causes are dermatophytes, demodex and bacterial folliculitis (typically Staphylococcal).
   - **Step 2:** examine the skin for clinical clues regarding hairshaft destruction e.g., broken hair shafts visible above the skin surface.
   - **Step 3:** examine clinically for a follicular oriented pattern: follicular casts, papules, pustules, comedones. Perform deep skin scrapings for demodex mites and trichograms for dermatophytes. Sample pustules for evidence of bacterial infection.
   - **Step 4:** proceed with other relevant screening tests or skin biopsy, as indicated.

2. Trichogram
   Hair shafts are readily available and some changes are definitely diagnostic! Hair loss can occur when the hairs are being broken off above the surface of the skin, when they are damaged within the hair follicle and when there is a developmental / growth problem or
complete lack of growth. I use trichoscopy on a daily basis but it does have its limitations.

A. Background information: the hair cycle. Hair cycles through 3 clinically relevant phases:
   a. The first: anagen, the growth phase, in which the hair bulbs are flexible, larger and when pigmented, the pigment extends down into the bulb. The duration of the anagen phase dictates the length of the hair shaft, so short coated animals have a shorter anagen phase than long coated animals.
   b. The second and third: catagen and telogen where the pigment begins to be reabsorbed from the living hair shaft (conservation of resources) so that the lower portion of the hair shaft will lack pigment and where the tiny interdigitating spikes of keratin become fixed and unmoving – resulting in a “spear-like” hair bulb which often has tiny spikes, or may be coated with a row of epithelial cells when plucked for trichoscopy (not to be confused with dermatophyte spores).

   The time from bulb to hair shaft appearing above the epidermis is approximately 6 to 8 weeks. Catagen is typically an extremely short phase in dogs and cats, but in plush coated dogs it may be prominent. Clipping a dog when there is a dominance of catagen hairs may result in the lack of hair growth we refer to as “post-clipping alopecia through a catagen arrest”. The telogen stage is the longest phase in the dog and cat. The hair shaft should stay in the hair follicle (so called “haired telogen”) until the new anagen hair growing from underneath “pushes” this out. However, if the hair shaft is lost, the follicle will stay empty (so called non-haired telogen) and this is the most common stage where we see alopecia.

   Anagen:telogen ratios in a trichogram are a useful tool in human medicine. In dogs, breed variation is frequently associated with haircoat differences. Poodles for example have approximately 90% anagen hair bulbs (similar to humans) associated with their long anagen cycle and as such a trichoscopy of a poodle with only 50% anagen hair shafts may support a suspicion of an endocrine aetiology.

B. Trichoscopy technique: Hair shafts need to be plucked using a specially coated forceps so that the hair shaft itself is not damaged. They need to be physically “yanked out” of the hair shaft, so that both the looser telogen hair shafts and the more firmly attached anagen hair shafts are both obtained. Sufficient numbers must be obtained to allow a diagnosis. An alternative to trichoscopy is to examine the hair shafts collected when one performs a deep skin scraping which may suffice for the diagnosis of for example anatomic hair shaft abnormalities.

C. Pros: Simple, easy, non-invasive and may confirm a diagnosis of hair shaft-associated abnormalities – including trichorrhexis nodosa, pigment associated alopecia – where pigment clumps large enough to distort the surface keratin may be visible, or dermatophyte spore infested hair shafts. Demodicosis may be easy to confirm - demodex mites may be identified attached to the hair shaft or bulb and also free within the microscopy field. The identification of mites on trichoscopy may be especially
helpful where a deep skin scraping is difficult, such as the anatomic location of periocular or ventral interdigital or with an aggressive dog.

Evaluation of hair bulb stage and anagen:telogen ratios: comparing number of telogen versus anagen hairs is useful in the case of 100% telogen hairbulbs. This is abnormal in all breeds and raises the possibility of a telogen effluvium. In poodles a predominance of telogen hairs can be used to support a suspicion of an endocrinopathy (not to make a diagnosis).

D. **Cons:** This technique relies on the number of hairs sampled and the selected sites and there are some specific facts that practitioners must remember:
   a. Dermatophytosis – if all of the infected hair shafts are already broken and not able to be “plucked” then the diagnostic spores on the hair shaft will be missed. In this case a deep skin scraping may actually yield a positive result.
   b. Demodicosis: trichoscopy was comparable to skin scrapings when an equivalent area of 1cm² was plucked. *One could question whether plucking 1cm² is more painful to the dog than a deep skin scraping?*
   c. Anagen:telogen ratios: Beagles have been reported with approximately 50:50 anagen:telogen ratio and arctic breeds with a lower ratio. No further work has been done in this field. The current use of anagen:telogen ratios in the dog is to be considered as “possibly helpful” rather than “very useful” and especially not “diagnostic” until we have a seasonally adjusted breed specific hair shaft development “map” to which to refer.
   d. When the aetiologic cause of the hair loss is hair follicle inflammation, there may be no abnormalities on trichoscopy: because the keratin is dead and fixed in shape, no hair shaft abnormalities result, the hair is simply prematurely shed.

E. **What do I do?** I use trichograms in every case of hypotrichosis and alopecia (at the edges). I pluck hairs from areas which are difficult to perform a deep skin scraping, such as periocular. When I cannot pluck hair shafts, I use a surface skin scraping to attempt to collect hair shaft remnants and pieces.

3. Polymerase chain reaction (PCR) test
   The **PCR test** takes a single copy or a few copies of a piece of DNA (DNA, cDNA, or RNA) and generates thousands to millions of copies. This multiplication is referred to as “amplification”. Quantitative PCR (qPCR / real-time PCR) is used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample, and has a very high degree of precision. qPCR methods use fluorescent dyes to measure the amount of amplified product in real time. PCR-based tests allow detection of small numbers of disease organisms (both live and dead). The use of PCR to detect disease organisms, especially those which are difficult or slow to be grown in the laboratory, can be extremely useful diagnostically. When developing a test, the diagnostic sensitivity (number of infected which test positive) and specificity (percentage without the disease which test negative) should be around 95% for both options. In addition, positive and
negative predictive values must be calculated – although this can be difficult for the less easy to culture forms of dermatophytosis.

A. Pros: Rapid and requires very little DNA to be present, so can detect very small numbers of organisms. For example in the case of Pox virus, the PCR test of a small piece of crust can replace the more invasive skin biopsy and histopathology.

B. Cons: Detects only the presence of (a tiny amount of) DNA, does not assess this as causative. If there is even a tiny amount of contamination of the specimen, this contaminant may be amplified leading to a false positive result. Diagnostic laboratories frequently select a subjective cutoff value for real-time amplification assays, above which a threshold cycle (Ct) value is deemed false. Commonly, higher cutoff values are interpreted as amplification or fluorescence artifacts, or cross contamination. This cut-off is the laboratories’ own choice.

4. PCR for dermatophytes
The internal transcribed spacer of ribosomal DNA (ITS), is the genome fragment targetted in most veterinary tests.

A. Pros: Rapid, sensitive. The current diagnosis of dermatophytosis, based on direct microscopy and culture of the clinical specimen, is problematic given the lacking specificity of the former and the length of time needed for the latter.

B. Cons: Dermatophyte PCR tests themselves are still in their infancy. In a review of 11 publications on the use of PCR tests for human dermatophytosis, Yvonne Graeser concludes “we are only at the beginning of providing high quality PCR diagnosis of dermatophytes”. In veterinary medicine the major problem we have is because the PCR test is sensitive. As dermatophyte DNA can also be found in the garden, how does one interpret a positive PCR test in an animal with no associated dermatophyte spores on hair shafts? And especially how does one interpret in an animal with no clinical lesions? (such as a kitten where the child has developed ringworm) The cat or dog may have picked up a small fragment of dermatophyte-DNA from the garden and may not even be what is termed a transient carrier.

C. What do I do? I have used a PCR for dermatophytosis where there are clinically compatible lesions and I will conduct a dermatophyte culture concurrently, in an attempt to obtain a more rapid result. I will have performed an extensive trichogram sampling in all cases.

5. PCR for demodex
Background: - Ivan Ravera established a real-time PCR technique and proved that demodex mites, albeit in very low numbers, were found to be normal inhabitants of haired areas of the skin of healthy dogs. He used hair samples (250-300 hairs with their hair bulbs) taken from five or 20 skin locations and a real-time PCR that amplified a 166 bp sequence of the D. canis chitin synthase gene. Demodex DNA was amplified from all 20 cutaneous points investigated, without statistically significant differences. He found that
the percentage of positive dogs increased with the number of sampling points. When a large canine population was sampled at five cutaneous locations, 18% of dogs were positive for demodex DNA. When 20 skin locations were sampled, all dogs tested positive for mite DNA. He concluded that demodex colonisation of the skin is present in all dogs, independent of age, sex, breed or coat.

A. *Pros:* Rapid, sensitive, could be useful in areas where skin scrapings are difficult to perform.

B. *Cons:* There are no publications to verify the use of this test as a diagnostic tool – a positive PCR of demodex DNA may be obtained in a case of bacterial folliculitis for example, because demodex mite DNA has been established to be identifiable on multiple sites in normal dogs. Ivan’s test was based on plucking 250-300 hairshafts with bulbs but one commercial laboratory currently runs this test from a skin scraping submission.

C. *What do I do?* I do not use the demodex PCR as a diagnostic test; I perform trichogram or deep skin scraping (with the pre-squeeze technique).

6. Thyroid testing - Resting T4 value

Hypothyroidism is reported to be the most common endocrine disorder of dogs, and up to 80% of cases result from autoimmune (lymphocytic) thyroiditis. It is heritable and so identification of hypothyroidism in a breeding animal is important.

A. *Pros:* This is an easy, inexpensive and readily available test. A resting T4 value in the higher 2/3rds of the normal reference range makes a diagnosis of hypothyroidism unlikely.

B. *Cons:* The definition of “normal” varies:

a. Laboratory factors: The normal thyroid range will vary from laboratory to laboratory and also with test type.

b. Animal factors:
   i. age / breed factors:
      1. Puppies have higher basal thyroid levels than adults
      2. Geriatrics have lower basal thyroid levels than adults
      3. Large / giant breeds have lower basal thyroid levels
      4. Sighthounds as a group have much lower basal thyroid levels
   ii. circulating hormones (oestrogen leads to lower T4 levels, progesterone to higher T4 levels), and
   iii. concurrent medication – e.g. corticosteroids, sulphonamides, iodine supplementation (kelp / seaweed) and phenobarbital all decrease measured resting total T4.

7. Thyroid testing- TSH

A. *Pros:* an elevated TSH value is supportive of hypothyroidism.

B. *Cons:* a large percentage of hypothyroid dogs can show a normal TSH value.
The cTSH test gives a 70% predictability for primary hypothyroidism in dogs vs 95% in people. Dogs regulate the pituitary-thyroid-hypothalamic axis via another pathway with growth hormone. Both false negatives and false positives occur.

A complete thyroid test should include most of the following:
T4, free T4, T3, free T3, TgAA (important if breeding or for breeds at risk for thyroiditis), T3 Autoantibody (T3AA) and T4 Autoantibody (T4AA).

8. Screening tests for Hyperadrenocorticism (Cushing’s syndrome) - Urine cortisol:creatinine (UCC) ratio
The amount of cortisol excreted in the urine is compared to the physiologically excreted creatinine in order to determine this ratio. Cortisol is not normally found in the urine in large amounts.
A. **Pros:** in a dog with no other systemic illness*, an elevated urine cortisol:creatinine ratio is a highly sensitive test. (*no clinical signs, no haematologic/serum biochemical abnormalities).

B. **Cons:** any stressed animal will secrete increased cortisol which can spill over into its urine. Adrenal neoplasia especially may secrete cortisol irregularly, so that a single sample may not detect a randomly secreting adrenal tumor.

Not a useful test for adrenal tumours in ferrets as these are typically associated with an overproduction of sex hormones, rather than cortisol.

Selected References
INTERPRETING BACTERIAL CULTURE AND SUSCEPTIBILITY REPORTS

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Introduction
Bacterial infections affecting the skin are part of the “day-to-day” life of small animal practitioners. Samples from skin and ear sites are most often submitted to the microbiology laboratory in order to identify a (or several) bacterial pathogens involved in infection and their antimicrobial susceptibility profile to guide treatment choices. Occasionally, swabs from carriage sites, such as the nasal and oral mucosa or perianal skin, are submitted to identify multidrug-resistant staphylococci such as MRSA or MRSP carried by a non-infected animal for example prior to an orthopaedic procedure or to help assess the risk of MRSA/MRSP spreading into the environment or to people. Whenever samples are submitted for bacterial culture and susceptibility testing, it is important to remember that the laboratory will only receive the organisms sent by us as clinicians and that the results need to be interpreted in the context of the patient’s history, clinical findings and ideally combined with cytology results in order to ensure a useful outcome for the patient. A swab from a dog’s skin, even from healthy skin, is highly likely to yield staphylococci and in the majority of dogs Staphylococcus pseudintermedius. The clinician will need to decide whether this result reflects infection or merely the dog’s cutaneous microbiota. In addition, it is the clinician’s responsibility to choose a laboratory experienced in working with bacterial pathogens isolated from animals. While semi-automated and automated methods, such as API® and Vitek® will have typically been designed for use in human microbiology laboratories, these can be used for veterinary diagnostics provided results are carefully interpreted by veterinary-trained microbiologists. Similarly, smaller veterinary diagnostic laboratories will need to keep up to date with the rapidly advancing methods and improvements, particularly in resistance testing, while practice in-house microbiology can no longer be recommended at a time when multidrug-resistant zoonotic pathogens have emerged and require accurate identification and careful handling.

Identification of staphylococci
Staphylococci are highly successful bacteria, colonising mammals and birds worldwide. They are robust on environmental surfaces surviving many adverse conditions for long periods and should therefore survive most transport conditions between the clinic and laboratory (suitable transport media still recommended for clinical samples). In the laboratory, staphylococci are easy to grow on many different nutrient agars and differentiation from other Gram positive cocci such as the micrococcii (usually by colony colour) and streptococci and enterococci (catalase test) is also straightforward. What is difficult though, is accurate species differentiation amongst staphylococci using phenotypic tests. Prior to the emergence of MRSA and MRSP as canine pathogens, species identification of coagulase-positive staphylococci isolated from a pet was of little relevance. Nowadays though, correct discrimination between S. aureus and S. pseudintermedius is critical in the context of methicillin-resistant isolates as epidemiology and zoonotic risk differ substantially between
MRSA and MRSP. MRSA, at least those isolated from pets, are predominantly of human-hospital origin and are better adapted to human hosts than to dogs or cats. Isolation of an MRSA will, therefore, prompt careful consideration of zoonotic aspects with an emphasis on human health. In contrast, MRSP is recognized as a veterinary nosocomial pathogen, well adapted to the canine host, and isolation should, therefore, initiate review of clinic hygiene procedures with an emphasis of preventing spread to other canine patients. Mistaken identities of *S. aureus* and *S. pseudintermedius* based on phenotypic testing occur (e.g. Börjesson et al. 2015). Despite a common belief that the differentiation between *S. aureus* and *S. pseudintermedius* in a microbiology laboratory is straightforward, this is by no means the case as non-pigment-producing *S. aureus* strains may be misidentified as *S. pseudintermedius* while the combination of multiple biochemical tests may eventually favour *S. aureus* as the more common species overall. It is, therefore, important to liaise with the laboratory in cases of methicillin-resistant isolates to ensure sufficient confirmative testing. Species identification by matrix-assisted laser desorption/ionization-time-of-flight analysis (MALDI-TOF) is becoming more widely accepted. This has substantially improved accuracy of species identification and in particular of differentiating between *S. aureus* and *S. pseudintermedius*. In order to discriminate further between the three species currently included in the *S. intermedius*-group (*S. intermedius*, *S. pseudintermedius*, *S. delphini*), although rarely needed for clinical purposes, MALDI-TOF may be less helpful and no accurate phenotypic tests are described either. Any isolate obtained from a dog with traditional phenotypic characteristics of *S. intermedius* can be assumed to be *S. pseudintermedius*, whereas isolates obtained from other species are best identified as bacteria of the ‘*S. intermedius* group’ (SIG) unless molecular test results are available.

**Other bacterial pathogens**

Involvement of *Pseudomonas aeruginosa* and other multidrug-resistant bacterial pathogens in skin and particularly ear infections will also prompt sample submission for bacterial culture and susceptibility testing. For *Pseudomonas spp.* susceptibility to fluoroquinolones, or at least to some compounds of the class, may still be reported. Resistance to fluoroquinolones is associated with point mutations in the DNA gyrase and topoisomerase genes and the occurrence of *in vitro* susceptibility to one but not all members of the class is still poorly understood. Where fluoroquinolone therapy is initiated for an isolate showing resistance to one or more other class members, close monitoring of treatment progress is warranted. For other multidrug-resistant bacteria that are currently emerging as major challenges in human hospitals such as *Klebsiella spp.*, *Escherichia coli* with extended-spectrum beta-lactamases (ESBLs), vancomycin-resistant enterococci (VRE), *Salmonella typhimurium* and *Acinetobacter baumanii* infections the challenge for clinicians will be initially to determine whether these occur as contaminants or pathogens. If deemed of clinical relevance, treatment may then have to be guided by minimum inhibitory concentrations (MICs) and using higher-end dosages of drugs as treatment options are often extremely limited. Similarly, identifying the most relevant pathogen amongst those reported from a mixed infection may be challenging and needs to be evaluated on a case-by-case basis.
Methicillin-resistance

MRSP (and to a lesser extent MRSA) can already be suspected from an antimicrobial susceptibility profile due to their extensive drug-resistance. However, testing specifically for methicillin-resistance as a marker for broad β-lactam resistance is indicated to allow correct allocation to the epidemiologically important group of methicillin-resistant staphylococci. In Europe at present, MRSP are typically resistant to all clinically relevant antibacterial agents including the fluoroquinolones, macrolides, and often also potentiated-sulphonamides (in addition to all β-lactams). Requesting extended susceptibility testing might be indicated for such isolates. MRSA isolated from small animals most often still show susceptibility to potentiated sulfonamides, tetracyclines and sometimes clindamycin. In vitro susceptibility to clindamycin needs to be interpreted with care though as inducible resistance is well recognized in staphylococci. The laboratory can be asked to test specifically for inducible resistance by D-testing to avoid inappropriate treatment choices.

Phenotypic testing for methicillin-resistance can be done either on screening agar or by disc testing and the chemically almost identical (but more stable) oxacillin is nowadays used instead of methicillin. For S. aureus in human medical laboratories, cefoxitin disc have replaced oxacillin as a better predictor for the presence of meca, the gene encoding broad β-lactam resistance. For S. pseudintermedius though, cefoxitin discs are currently not recommended by CLSI. Confirmation of the meca gene by polymerase-chain reaction (PCR) or of its product (a mutated penicillin-binding protein in the cell wall) by latex agglutination is commonplace in medical laboratories and for research purposes but not in veterinary diagnostic laboratories. An important area where clinicians need to interpret the laboratory report carefully is the broad β-lactam resistance associated with meca-positive isolates. If meticillin-resistance is reported, resistance should be assumed to all β-lactams, including the first and the veterinary third-generation cephalosporins, irrespective of what is reported. Confusion may occur for example over a meticillin-resistant S. pseudintermedius isolate reported as susceptible to amoxicillin-clavulanate. This in vitro susceptibility will not translate into in vivo efficacy and all beta-lactams should be avoided. Reasons for such irregularities are still unclear but breakpoints are currently reviewed.

Susceptibility testing

Testing for antimicrobial susceptibility is commonly done by (modified) Kirby-Baur disc diffusion tests. Alternatively (or in combination), some laboratories determine an isolate’s minimum inhibitory concentrations (MICs) through the more labour-intense agar dilution or broth dilution method or by E-strip testing (paper strips impregnated with increasing concentrations of antimicrobial). Breakpoints are used to categorize microorganisms as clinically susceptible (S), intermediate (I) or resistant (R) dependent on the quantitative antimicrobial susceptibility as indicated by the MIC value determined in a well-defined standard test system and defined procedures. Categories S, I and R should help to distinguish between patients that are likely or unlikely to respond to antimicrobial treatment. Breakpoints are determined by breakpoint committees composed of specialists in clinical trial science and in pharmacokinetics and pharmacodynamics, population simulation tools, resistance mechanisms, antimicrobial susceptibility testing methods and bacterial population dynamics. Two such committees are the Clinical and Laboratory Standards Institute of the United States of America (CLSI) and the European Committee on Antimicrobial Susceptibility Testing
(EUCAST) and both include documents on breakpoints for veterinary pathogens. Use of such agreed breakpoints ensures best available data is included in predicting treatment success and standardised breakpoints will be the basis for useful surveillance data. Knowledge of actual MICs is rarely required for skin infections but more relevant for serious infections such as bacteraemia involving multidrug-resistant bacteria where increasing the dose of an antimicrobial for an intermediate resistant isolate for example may be preferable to choosing a more toxic drug.

A special considerations with regard to susceptibility testing of skin and ear pathogens is that susceptibility test reports are relevant if systemic antibacterial therapy is to be used but less so for topical therapy. For example, a *Pseudomonas spp.* isolated from a dog’s ear swab and reported as resistant to enrofloxacin or marbofloxacin based on *in vitro* testing is very likely to be sensitive to topical therapy with these agents as concentrations achieved at the site of infection through ear drop application are expected to exceed MICs by far. Clinical breakpoints are designed to help predict efficacy using systemically administered drugs, not topical therapy.

**Summary**

Empirically chosen systemic antibacterial therapy is increasingly hampered by the continuing spread of multidrug-resistance amongst bacterial pathogens. While phenotypic microbiology testing will always be challenged by strain variation, thorough laboratory testing and correct interpretation of results will be critical for patient management. Careful interpretation of species identification and of reported susceptibility results and communication with experienced veterinary diagnostic laboratories is essential so that additional tests can be initiated in some cases to improve certainty and hopefully treatment outcome.

**Selected References**

CUTANEOUS MANIFESTATIONS OF SYSTEMIC DISEASE

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1. Introduction

While not always thought of as an organ, the skin is in fact the largest and most visible organ of the body. Cutaneous markers of internal diseases are common and are often highly indicative for a specific systemic disease. Recognition of the significance of cutaneous symptoms facilitates diagnosis of underlying disease processes.

2. Systemic Diseases Associated with Alopecia

The classical systemic diseases associated with generalized alopecia are the endocrinopathies, however it is important to rule out other causes of generalized alopecia.

A. Hypothyroidism. Thyroid hormones are necessary for the initiation of the anagen phase of the hair follicle cycle. In a hypothyroid animal, hairs remain in the telogen phase until shed.

B. Hypercortisolism (Hyperadrenocortisim). The skin is a sensitive indicator of hypercortisolism with both non-specific (alopecia, secondary infections, poor wound healing) and specific findings (thinning of skin, calcinosis cutis, phlectasias, striae).

C. Hyperestrogenism. Hyperestrogenism may develop in female dogs as a result of polycystic ovaries or ovarian neoplasia. Cutaneous signs include bilaterally symmetric alopecia, enlarged vulva and nipples, and a history of abnormal estrus cycles. Male dogs develop hyperestrogenism as a result of an estrogen-secreting testicular tumor (most commonly a Sertoli cell tumor, particularly common in cryptorchid testes). Both sexes can develop iatrogenic hyperestrogenism. A unique cutaneous manifestation of hyperestrogenism in male dogs is linear preputial dermatosis – a linear, erythematous or hyperpigmented narrow band of skin located between the prepucial orifice and the scrotum.

D. Pituitary Dwarfism. Pituitary dwarfism has been reported as an autosomal recessive disorder in the German shepherd dog and Carnelian bear dog.

3. Systemic Diseases with Diffuse Hypotrichosis, Scales and Crusts

A. Feline Thymoma-Associated Exfoliative Dermatitis. Skin lesions of erythema, crusting and scaling with alopecia may be the first sign of disease in cats with thyomas. Secondary infections with Malassezia may result in pruritus.

B. Epitheliotropic/Cutaneous T-Cell Lymphoma. Epitheliotropic cutaneous lymphoma can present with a variety of cutaneous manifestations including erythroderma, alopecia, variable pruritus, scales, crusts, plaques, nodules, leukoderma, mucocutaneous ulcers, and/or depigmented, ulcerated or hyperkeratotic footpads.
4. Systemic Diseases with Facial and Dorsal Erythema, Alopecia, Scaling and Crusting
   A. *Leishmaniasis.* A characteristic finding in animals with leishmaniasis is silvery white scales over the head, pinnae and limbs. Nasodigital hyperkeratosis may also be present. Periocular alopecia is common. Other findings include onychogryposis, paronychia, nasal depigmentation and nodular dermatitis.
   B. *Lupus Erythematosus.* Lupus erythematosus is an autoimmune, inflammatory, multisystemic disorder of complex etiologies that may have clinical manifestations restricted to the skin or may involve multiple organs.
      i. Discoid lupus erythematosus (DLE). DLE is generally restricted to skin lesions affecting the face, particularly the planum nasale and dorsal surface of the nose.
      ii. Systemic lupus erythematosus (SLE). SLE is a multisystem disease with variable clinical presentations. Skin lesions are usually symmetrical and vary from mild scaling and/or alopecia of the face and dorsum to severe ulcerations.
      iii. Exfoliative Cutaneous Lupus Erythematosus (ECLE). ECLE is a familial form of lupus affecting German shorthaired pointer dogs. Skin lesions are often the first abnormality noted and include excessive scaling and crusting of the muzzle, pinnae and dorsum.

5. Systemic Diseases with Alopecia, Erythema, Scales and Crusts Affecting Face, Legs and Tail
   *Dermatomyositis (DM).* DM is an inflammatory disease affecting skin and/or muscle. In dogs, DM occurs most often in collies and Shetland sheepdogs, although other breeds have also been affected. Skin lesions vary from mild to severe and start with small focal areas of crusting, scaling and alopecia, most commonly affecting the face, lower extremities and tail. Over time the skin becomes atrophic.

6. Systemic Diseases with Alopecia and Shiny Skin of Periocular Region, Ventral Neck, Ventral Abdomen and Legs
   A. *Feline Paraneoplastic Alopecia.* A rapid development of extensive alopecia in an older cat may be the first symptom of underlying pancreatic or hepatic neoplasia. Secondary *Malassezia* infections may result in excessive grooming. Some cats also have dry, scaly footpads.

7. Systemic Diseases with Cutaneous Erythema, Alopecia, Scales and Crusts of Mucocutaneous Junctions and Footpads
   A. *Zinc-Responsive Dermatitis.* Skin lesions associated with zinc-deficiency or zinc-responsive dermatitis include areas of erythema, alopecia, scales and crusts starting on the face, mucocutaneous junctions, feet and footpads.
   B. *Lethal Acral Dermatitis/Acrodermatitis.* Bull terriers may be affected by an autosomal recessive condition associated with defective zinc and copper metabolism and altered production of 13 liver proteins. Affected puppies have lighter than normal skin pigmentation and often have difficulty in chewing and swallowing. They develop splaying of their digits, crusting and cracking of their footpads and ulcerated crusted
lesions on their ear pinnae and mucocutaneous junctions. Foot lesions progress to onychodystrophy, paronychia and interdigital pyoderma.

C. **Canine Distemper.** The classical skin manifestation of canine distemper is “hard pad disease” with hyperkeratosis of the footpads and planum nasale.

D. **Superficial Necrolytic Dermatitis (SND) (Metabolic Epidermal Necrosis, MEN) (Hepatocutaneous Disease) (Necrolytic Migratory Erythema, NME).** NME in humans is a cutaneous marker for alpha2-glucagon-producing pancreatic islet cell tumors. In dogs only a low proportion of dogs with classical lesions of SND have underlying glucagonomas, the majority of canine cases are diagnosed with liver disease. Lesions start in areas of high cell turnover including mucocutaneous junctions, face, footpads and pressure points. Skin lesions include crusts and ulcerations with perilesional erythema. The disease in cats has been associated with pancreatic tumors and chronic liver disease.

E. **Paraneoplastic Pemphigus.** Paraneoplastic pemphigus has been reported in dogs in association with thymic lymphoma and splenic sarcoma.

8. **Systemic Diseases with Cutaneous Plaques**
   A. **Calcinosi Cutis.** Calcium deposits form granular plaques in the skin, most commonly over the dorsum or in the inguinal region, as a result of apatite crystals deposited in the dermis, usually in association with collagen or elastin fibers. Differential diagnoses include hypercortisolism, renal disease, blastomycosis, paecilomycosis and other inflammatory diseases.
   
   B. **Xanthomas.** Xanthomas appear as yellow-white papules, nodules or plaques located on the head, limbs, feet or over bony prominences. These lesions are cutaneous manifestations of abnormalities in lipid metabolism or consumption of extremely high-fat diets.
   
   C. **Hyperpigmented Viral Plaques.** Canine viral plaques are an uncommon manifestation of papillomaviral infection in dogs.
   
   D. **Eosinophilic Plaques.** Eosinophilic plaques develop in cats in association with underlying allergies (parasitic, dietary or environmental). Differential diagnoses include mast cell tumor, squamous cell carcinoma, lymphoma and metastatic mammary adenocarcinoma.
   
   E. **Cutaneous Lymphoma—Plaque Form**

9. **Systemic Diseases with Cutaneous Nodules**
   A. **Nodular Dermatofibromas.** German shepherds and related dogs have an autosomal dominant genetic disease caused by mutations in the folliculin gene (FLCN) that results in the development of multiple cutaneous nodules and multiple cysts and cystadenocarcinomas in the kidneys. Female dogs may also develop uterine leiomyomas and leiomyosarcomas.
   
   B. **Sterile Nodular Panniculitis (SNP)—Pancreatic Panniculitis Variant.** Sterile nodular panniculitis is characterized by sterile subcutaneous inflammatory nodules that may ulcerate and drain a purulent or oily exudate. Lesions often develop in the absence of any other known specific diseases or infectious agents, however pancreatitis and pancreatic tumors have been associated with panniculitis.
C. *Infectious/Pyogranulomatous Nodules*. Cutaneous nodules develop in many animals with blastomycosis, coccidiomycosis, cryptococcosis, histoplasmosis and aspergillosis.

10. Systemic Diseases with Skin Fragility
   A. *Cutaneous Asthenia (Ehlers Danlos Syndrome, Dermatosporaxis)*. Cutaneous asthenia is a hereditary disease with defective formation of connective tissue resulting in skin fragility and, in some cases, joint hyperlaxity, hernias and vascular complications.
   B. *Feline Skin Fragility*. Acquired skin fragility is most often seen in cats with hypercortisolism secondary to adrenal tumors or iatrogenic administration of glucocorticoids or progestational compounds.

11. Systemic Diseases with Thick Skin
   A. *Mucinosis*. The skin may be thicker than normal due to excessive amounts of mucin in the dermis as a breed-associated characteristic of Chinese Shar-Pei dogs or as a result of hypothyroidism.
   B. *Acromegaly*. Cutaneous changes associated with acromegaly include thickened myxedematous skin with excessive skin folds, hypertrichosis and thick hard claws.

12. Summary
    Changes in the appearance of the skin and hair coat provide important clues for the diagnosis of a number of systemic diseases. Other differentials should always be ruled out, skin biopsies are helpful in the diagnosis of many of these diseases.

Selected References


GENETIC SKIN DISEASES IN DOGS

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Introduction
The molecular genetics of several canine genodermatoses has recently been solved. The dog represents the species with the largest number of genodermatoses with known genetic etiology among domestic animals. A brief review of selected genetic findings of the last ~4 years will be given, followed by a compilation of some other genodermatoses whose molecular genetics is not yet solved.

Genodermatoses with Known Genetic Cause

Ectodermal dysplasia - skin fragility syndrome (ED-SFS) in Chesapeake Bay Retrievers (OMIA 001864-9615)
ED-SFS affected puppies typically die within a few days after birth due to their severe skin lesions. With special care and avoidance of any mechanic stress to the skin, puppies can be kept alive for a few months, but the poor prognosis warrants euthanasia once the diagnosis is established. ED-SFS is caused by a splice site variant in the \textit{PKP1} gene encoding plakophilin 1 (c.202+1G>C). It is inherited as a monogenic autosomal recessive trait. There is a homologous disease in humans and the Chesapeake Bay Retrievers with ED-SFS were proposed as potential model to evaluate potential treatments for the human disease (Olivry \textit{et al.} 2012). Genetic testing is available and is recommend as a diagnostic tool in questionable ED-SFS cases and to avoid accidental carrier x carrier matings by dog breeders.

Footpad hyperkeratosis in Irish Terriers and Kromfohrländer dogs (OMIA 001327-9615)
Footpad hyperkeratosis, also termed hereditary footpad hyperkeratosis (HFH) or digital hyperkeratosis (DH) is an autosomal recessive disease. The predominant clinical sign are fissures in all four paw pads, typically starting at an age of 4-6 months. If not properly managed, these fissures can get very deep. Secondary bacterial infections may complicate the disease and cause severe pain to the dog. With adequate care, affected dogs have a good quality of life and normal life expectancy. Affected dogs also have a characteristic coat appearance that is coarser and less regular as in non-affected dogs.

Footpad hyperkeratosis is caused by a missense variant in the \textit{FAM83G} gene, encoding family with sequence similarity 83 member G, a protein with unknown biochemical function. The phenotype in \textit{FAM83G} mutant dogs and mice suggests a role in maintaining the homeostasis of the palmoplantar epidermis and an influence on hair growth (Drögemüller \textit{et al.} 2014).
Keratoconjunctivitis sicca and ichthyosiform dermatosis (CKSID) in Cavalier King Charles Spaniels (OMIA 001683-9615)

Congenital keratoconjunctivitis sicca and ichthyosiform dermatosis (CKSID) or “dry eye curly coat syndrome” is inherited as a monogenic autosomal recessive trait. Affected puppies suffer from severe eye infections as soon as their eyes open in addition to changes in the coat and footpads. The severity of the eye problems warrants euthanasia.

Interestingly, CKSID is caused by a frameshift variant in the \textit{FAM83H} gene \cite{Forman2012}. CKSID is thus genetically closely related to footpad hyperkeratosis, which is caused by a variant in the \textit{FAM83G} gene. The \textit{FAM83} gene family has eight members (\textit{FAM83A} – \textit{FAM83H}), and so far very little is known about the biochemical or physiological functions of the encoded proteins. Based on the findings in \textit{FAM83G} and \textit{FAM83H} mutant dogs, it is tempting to speculate that the other members of this gene family may also play some role in maintaining the integrity of the epidermis.

Ichthyosis in the American Bulldog (OMIA 001980-9615)

In the American Bulldog, this genodermatosis has also been termed autosomal recessive congenital ichthyosis (ARCI). The gross phenotype is manifest as a disheveled pelage shortly after birth, generalized scaling, and adherent brown scale with erythema of the abdominal skin \cite{Mauldin2015}. ARCI in American Bulldogs is caused by a yet unpublished variant in the \textit{NIPAL4} gene \cite{Casal2015}. Genetic testing is available at the University of Pennsylvania.

Ichthyosis in Golden Retrievers (OMIA 001588-9615)

Golden Retriever ichthyosis is a frequent genodermatosis in this breed. It is inherited as a monogenic autosomal trait. The clinical signs are very mild and sometimes not even noticed by the dogs’ owners. The causative genetic lesion is a complex frameshift variant in the \textit{PNPLA1} gene and the discovery of this variant in dogs subsequently helped to identify comparable \textit{PNPLA1} variants in human patients with unsolved ichthyoses \cite{Grall2012}.

The most challenging aspect of this genodermatosis are the consequences for breeding. Immediately after the introduction of genetic testing, the genotype frequencies in Golden Retrievers from Switzerland were 32% affected (mut/mut), 49% carriers (mut/+), and only 20% clear (+/+). It would be detrimental to the breed, if all carriers and affected Golden Retrievers were excluded from breeding as this would concern 80% of the population. Breeders should be counseled to implement a sustainable selection against this allele. Matings with at least one clear dog are optimal to avoid the birth of affected puppies. However, as the disease has a very mild clinical phenotype, it may be preferable to produce a few more affected dogs rather than now excessively using the minority of clear Golden Retrievers for breeding. A frequent use of a small number of dogs with subsequent intense inbreeding would be highly likely to provoke other, and potentially more severe, recessive defects.

Ichthyosis in the Great Dane (OMIA 001973-9615)
Ichthyosis in Great Danes is characterized by a generalized severe hyperkeratosis with formation of a strongly wrinkled, thickened and scaling skin, especially in the region of the eyes and nose. These changes lead to a dry inelastic and lichenified skin of an untidy appearance in the affected dogs and a markedly swollen periocular skin which impedes the opening of the puppy’s eyes in some cases. In-between the wrinkles the exudative character of the skin promotes secondary infections. Due to the poor prognosis, all affected dogs should be euthanized. The disease follows a monogenic autosomal recessive inheritance and is caused by a variant in the \textit{SLC27A4} gene encoding solute carrier family 27 (fatty acid transporter), member 4 (Metzger et al. 2015).

\textit{Nasal parakeratosis in Labrador Retrievers (OMIA 001373-9615)}
Nasal parakeratosis, also called hereditary nasal parakeratosis (HNPK), is another genodermatosis with monogenic autosomal recessive inheritance. Clinical signs include fissures and crusts on the nasal planum. Secondary bacterial infections can cause additional pain to the dog. HNPK is caused by a missense variant in the \textit{SUV39H2} gene (c.972T>G; p.N324K). The \textit{SUV39H2} gene encodes a histone methylase and is thus involved in the epigenetic modulation of gene expression during keratinocyte differentiation (Jagannathan et al. 2013). Genetic testing is available and is recommend as a diagnostic tool in questionable HNPK cases and to avoid accidental carrier x carrier matings by dog breeders.

\textbf{Genodermatoses & Hair Morphology Traits with Unknown Genetic Etiology}
Dermatomyositis in Collies and other herding dogs, complex inheritance
Hairlessness in the American Hairless Terrier, monogenic autosomal recessive
Lethal acrodermatitis in Bull Terriers, monogenic autosomal recessive

\textbf{Multifactorial Dermatologic Conditions with a Suspected Genetic Influence}
Alopecia X (Pomeranian and other Spitz breeds)
Atopic dermatitis
Bald Thigh syndrome (Greyhound)
Follicular dysplasia (Curly Coated Retriever)
Sebaceous adenitis (Standard Poodles and Akitas)
Symmetrical lupoid onychodystrophy (SLO)

Selected References

Introduction
Flea control has evolved tremendously over the last decade. There are a variety of excellent traditional and new forms of flea-control products giving veterinarians and animal owners many options when it comes to choosing topical and oral products. Selection depends on a variety of factors including budget, toxicity, bathing frequency, preference of topical vs oral administration and, of course, efficacy. In regards to efficacy, both residual speed of kill and duration of activity have become very important factors when it comes to controlling fleas and, in particular, managing pets with flea allergic dermatitis.

Insect growth regulators (IGRs) and insect development inhibitors (IDIs)
These products are very safe and effective for both environmental flea control and for topical animal use. Methoprene and pyriproxifen are the two currently marketed IGR’s in the U.S. and Europe. The current IDI is lufenuron (Program® and Sentinel®: Elanco/Novartis). These agents are administered orally, by injection (lufenuron) or topically (pyriproxifen, methoprene), and provide long-term (usually 30 days or longer) ovicidal and larvicidal effects. Methoprene is quickly inactivated by ultraviolet light, which is not the case with the other two. All IGRs are analogues of the insect juvenile hormone. Juvenile hormone regulates larval DNA transcription, maintaining larval information. Other hormones then trigger the fall in the level of juvenile hormone, which allows DNA that codes for adult characteristics. Thus, when the apparent level of juvenile hormone does not fall, pupation cannot occur. This leads to very large larvae, which generally die, or to abnormal pupae, which cannot hatch. They are also ovicidal. Lufenuron is a benzoyl urea that inhibits chitin development if present in blood ingested by the flea. It interrupts embryogenesis, hatching and molting. The fleas are unable to exit from the egg. Pyriproxifen (Nylar®) has the longest action, is the most stable, and remains 100% effective for 150 days after a single spray application.¹ It is an excellent option in cases when Program® may be too expensive because of a multiple-pet household or in situations where Program® is ineffective. It is presently available as a topical spot-on in combination with permethrin for dogs in the Vectra® line. Environmental foggers and sprays are also being marketed and many professionals use this chemical for home treatments.

Fipronil-based products
Frontline Plus® and Frontline Top Spot®: Merial Animal Health
Parastar™ and EasySpot™: Elanco/Novartis
Many generic fipronil products available
Certifect™ (amitraz-methoprene): Merial Animal Health
Frontline Tritak (cyphenothrin): Merial Animal Health
Fipronil is a phenylpyrazole, which blocks the action of GABA – an essential neurotransmitter. Frontline plus contains the insect growth regulator S-methoprene. These
products are available as spot-on formulations: Frontline and Frontline plus (9.7%, Merial Animal Health), Parastar and EasySpot (Elanco/Novartis), Certifect (also contains amitraz-methoprene, Merial Animal Health), Frontline Tritak (also contains cyphenothrin, Merial Animal Health), and many generic products. It has previously been reported to have a month’s activity with 95% or greater removal of fleas. However, newer studies suggest potential concerns for resistance and lower success has been seen in many studies. Immersion in water has little effect, as it is lipid soluble. It is also effective against ticks, sarcoptes and cheyletiella, although it has a somewhat slow action. It is very safe with less than one adverse reaction in 200,000 doses. Although not labeled to be used more frequently, some investigators use at 2-3 week intervals for more complete control in flea allergic animals. There are two new Merial fipronil-based products: Certifect that contains methoprene and amitraz is more of a tick control product and cannot be used in cats due to the amitraz. The second product is Frontline Tritak, which contains cyphenothrin that aids in the speed of kill. It is also important to note that some of the generics that are available over-the-counter do not contain the same purified forms of fipronil or lack methoprene that is found in the Merial products. Because of its diminished efficacy, fipronil is being prescribed much less by veterinarians but the over-the-counter market continues to make fipronil a commonly used agent.

**Imidacloprid-based products**

*Advantage® K9 Advantix® (permethrin), Advantage Multi® and Advocate® (moxidectin): Bayer Animal Health*

These products are available as topical spot-on treatments for either dogs or cats. Imidacloprid is the common ingredient in all of these products and is a chloronicotyl nitroguanidine that acts by binding to the nicotinergic receptors in the postsynaptic nerve region, thus preventing binding of acetylcholine. It acts for approximately the same length of time as fipronil, but is water soluble and more readily removed by water. It has no effect against ticks and no apparent flea repellent effects unless the combined product (imidacloprid and permethrin - Advantix) is used. Advantage Multi and Advocate are prescription drugs that are also heartworm preventives (moxidectin) and have efficacy for other ectoparasites including scabies and cheyletiella, with some reports showing efficacy for demodicosis. Imidacloprid when applied at its target dosage of 10mg/kg achieved 86.6% flea control within 6 hours and up to 97.6% within 12 hours. Efficacies are maintained between 97.8% and 100% for dogs and between 90% and 96% for cats through day 28. The product appears to be affected by water and shampooing, and should be reapplied every 2 weeks if frequent bathing is performed.

**Selamectin-based products**

*Revolution®: Zoetis Inc*

Selamectin is a semi-synthetic avermectin, derived from doramectin. It is available as an isopropanol/dipropylene glycol mono-methyl ether-based topical liquid (6% or 12 % w/v active) for spot-on application in both dogs and cats. It is also effective against ticks, sarcoptes, cheyletiella, heartworm prevention, otodectes and some internal parasites. Its efficacy against fleas in both dogs and cats was greater than 90% after the first month of administration and exceeded 99% on day 90 following three consecutive monthly
applications. It appears that selamectin is fairly water-resistant as long as bathing or water immersion is delayed for at least 2 hours after application. The author has also used this product safely on an every 2-week basis without adverse reactions. Selamectin has been shown to be as effective as fipronil and imidacloprid in reducing *C. felis* infestation in dogs housed for 3 months in a flea-infested environment. The safety of selamectin is well established and additional studies have been performed in ivermectin – sensitive collie dogs establishing its safety in this breed. The author tends to use this product more in situations where flea control and additional parasite management are indicated. It is also effective and well tolerated in cats.8

**Nitenpyram-based products**

Capstar®: Elanco/Novartis

Nitenpyram is at neonicotinoid flea adulticide. It is chemically similar to imidacloprid, but differs as it is orally administered at a minimum target dose of 1mg/kg. Activity is rapid in onset, but short acting. It removes over 95% of adult fleas from dogs and cats within 4 – 6 hours of oral administration and has residual activity for 48 -72 hours. It is very safe to use in both dogs and cats.9 When used 2 -3 times a week with monthly lufenuron, excellent control flea control has been accomplished. Nitenpyram can also be used for situations of high-risk flea exposure as a one or two-time treatment (e.g., kenneling, dog or cat shows, trips to the veterinarian or groomers, dog parks, etc).

**Spinosad and Spinetoram-based products**

*Spinosad - Comfortis® for Dogs and Cats and Trifexis (with milbemycin) for Dogs: Elanco*

*Spinetoram - Cheristin® for Cats: Elanco*

The spinosad-based products are only approved for use in the dogs and have a novel mode of action at nicotinic acetylcholine D alpha-receptors with some effects on GABA resulting in nerve excitation paralysis and death of the flea. Spinosad is felt to be safe in conjunction with all other flea control products and heartworm preventives. There is one interaction that has recently been noted — spinosad can increase the risk of ivermectin side effects when ivermectin is used at the very high doses required to treat skin parasites such as demodicosis. Low doses of ivermectin as used in heartworm prevention are not problematic for this interaction. It does not kill other internal or external parasites and is for dogs 14-weeks of age and older. Comfortis tablets are beef flavored but contain pork protein and hydrolyzed soy. That should not be a problem for dogs with beef allergy but could be a problem for a dog with a pork or soy allergy. It is highly effective, starts working within 30 minutes, has 100% kill rate by 4 hr and lasts 30 days or longer. It is best given with food for the highest C max and longest duration of effectiveness. Because it kills fleas so rapidly, egg formation does not occur. Clinical trials in dogs have shown excellent results for not only flea control but in flea allergic dogs with reduced clinical symptoms.10 Most studies look at laboratory evaluations of products where investigators administer the products directly and create an artificial environment that does not typically replicate what veterinarians see in their practices. However a recent study performed by Dryden in clinical “real world” evaluation compared spinosad (SPN) to fipronil and methoprene (FSM). This study showed what to expect from either product in true clinical settings when the products are dispensed for use at
home and applied or given by the owner. What was most impressive from this study was not only the reduction in flea numbers (55/58 (95%) SPN vs 21/55 (38%) FSM at day 90, but the reduction in the clinical scoring of pruritus based on a previously validated pruritus scoring system. Following the criteria as established by this validated pruritus scoring system, a score of less than 2/10 supports a “normal” level of pruritus. In this study, the spinosad-treated dogs achieved this “normal” level in over 90% of the cases by day 90 compared to only 49% of the fipronil/methoprene treated dogs. This reduction in pruritus and excellent efficacy regarding speed-of-kill and duration of activity makes spinosad a very commonly prescribed form of flea control in my practices.

Spinetoram (Cheristin: Elanco) is the newest topical flea control for cats and kittens 8 weeks of age or older. It replaces Assurity, which was the previous feline spinetoram product; although it was highly effective, the Assurity product was associated with localized topical reactions. These reactions were thought to be due to a combination of high concentration of spinetoram (39%) and a benzyl alcohol vehicle. The new product Cheristin has a lower concentration of spinetoram (11%) and no benzyl alcohol, eliminating any significant topical reactions but maintaining excellent efficacy. It starts killing fleas within 30 minutes and kills 100% of fleas within 12 hours with 99% flea control for a full month. Trifexis has the added benefit of milbemycin giving additional prevention of heartworm infection and treatment and control of adult hookworm, adult roundworm and adult whipworm infections.

Dinotefuran-based products

Vectra 3-D for Dogs® (dinotefuran, permethrin, pyriproxifen: Summit Vet Pharm/Ceva)
This product is a monthly spot-on application for flea, tick and mosquito control with an insect growth regulator. It contains 4.95% dinotefuran (flea adulticide), 0.44% pyriproxyfen (IGR) and 36% permethrin (tick, mosquito & flea parasiticide/repellent). It has a quick kill (96% of fleas within 6 hours). Like all topical products, efficacy decreases when the animal is bathed. Water and shampooing can lower efficacy after 14 days. It does contain a large dosing volume (large dog-8mL) and needs to be applied in multiple sites or spread along the entire dorsal surface of the body. Care needs to be taken to avoid inadvertent application of the canine product on cats, as permethrin is toxic to cats and extra care should be used when prescribed in households where cats are present. Dinotefuran is a third-generation neonicotinoid that binds permanently to insect nAChR- gated Na+ channels leading to tremors, uncoordinated movement and death. It kills by contact and ingestion is not necessary, thus this is very beneficial for flea allergic animals. Vectra 3D successfully inhibited flea feeding immediately after flea infestation, with overall reduction in feeding of 79% within the first hour after application compared to non-treated controls. Results of this flea feeding inhibition were seen as quickly as 5 minutes after fleas were placed on treated dogs.

Vectra for Dogs and Puppies® (dinotefuran and pyriproxifen): Summit Vet Pharm/Ceva
This product does not contain permethrin but has a higher concentration of dinotefuran (22%) and pyriproxifen (3%), designed for pure flea control. It would be indicated in cases of FAD where rapid kill is critical for the control of pruritus in the hypersensitive patient. Studies
have shown that both of the above mentioned dinotefuran topical formulations were highly effective against flea-infested dogs throughout a study period of 28 days post treatment.\textsuperscript{13}

*Vectra for Cats\textsuperscript{®} (dinotefuran, pyriproxifen):* Summit Vet Pharm/Ceva
This product is a monthly spot-on application for flea, tick and mosquito control with an IGR. It provides long-lasting repellency, and is a fast acting flea adulticide that also provides control for the egg stage of the flea for at least 30 days. It does not contain permethrin and is safe for use in cats.

**Indoxacarb-based products**
*Activyl\textsuperscript{®} and Activyl Tick Plus\textsuperscript{®}:* Merck Animal Health
Activyl is the first flea control product to utilize metabolic activation, or bioactivation of the active ingredient, to kill fleas. It is a topical, once-a-month ectoparasiticide containing indoxacarb, a compound never before used in animal health, with no known resistance in fleas. Following application, indoxacarb enters the flea by contact or ingestion and is converted by enzymes in the flea to an active highly insecticidal metabolite. Fleas stop feeding, become paralyzed and die within hours. Activyl not only kills adult fleas, but also disrupts the flea’s life cycle by inhibiting the development of flea larvae in the pet’s surroundings. This aids in the environmental control of flea infestation and helps prevent re-infestation for up to a full month. In laboratory studies, Activyl was shown to start killing fleas within hours of application and to keep killing newly arriving fleas within 8-12 hours for four weeks. In field studies, Activyl outperformed fipronil-containing products, making more dogs and cats flea free.\textsuperscript{15,16} It has also been shown to achieve complete resolution of clinical signs of FAD in 21/25 cases (87.5\%) with nearly complete resolution or marked improvement in the remaining animals.\textsuperscript{17} Activyl Tick Plus is exclusively for dogs and combines the effective flea control of Activyl (indoxacarb) with long-lasting protection against ticks (permethrin).

**Isoxazolines**
*Afoxolaner- NexGard\textsuperscript{®}:* Merial Animal Health
Afoxolaner is a member of the isoxazoline family, shown to inhibit insect and acarine ligand-gated chloride channels, in particular those gated by the neurotransmitter gamma-aminobutyric acid (GABA), thereby blocking pre- and post-synaptic transfer of chloride ions across cell membranes. Prolonged afoxolaner-induced hyperexcitation results in uncontrolled activity of the central nervous system and death of insects and acarines. The selective toxicity of afoxolaner between insects and acarines versus mammals may be inferred by the differential sensitivity of the insects and acarines' GABA receptors versus mammalian GABA receptors. In a well-controlled laboratory study, Nexgard demonstrated 100\% effectiveness against adult fleas 24 hours post-infestation for 35 days, and was ≥ 93\% effective at 12 hours post-infestation through day 21, and on day 35. In a 90-day U.S. field study conducted in households with existing flea infestations of varying severity, the effectiveness of Nexgard against fleas on the Day 30, 60 and 90 visits compared with baseline was 98.0\%, 99.7\%, and 99.9\%, respectively.
Collectively, the data from the two studies (one laboratory and one field) demonstrate that Nexgard kills fleas before they can lay eggs, thus preventing subsequent flea infestations after the start of treatment. Another more recent study looked at naturally infested dogs in private residences in Tampa, Florida. Evaluations of on-animal and premise flea burdens, flea sex structure, and fed-unfed premise flea populations were conducted. Dogs and environmental sampling occurred on days 0, 7, 14, 21, and once between study days 28-30, 40-45, and 54-60. Within 7 days of afoxolaner administration, flea counts on dogs were reduced by 99.3%. By one month post-treatment, flea counts were reduced by 99.9%, with 97.3% (36/37) of the dogs being flea free. Following the second dosing on study day 28-30, total on-dog flea burden was reduced by 100% on days 40-45 and 54-60. Reductions in emerging flea populations were 97.7 and 100% by days 28-30 and 54-60, respectively. Seven days after initial treatment, only 13.1% of the fleas contained blood and by day 14 only 4.9% of the fleas collected in traps displayed evidence of having fed. On day 0, prior to treatment, 60% of the unfed fleas collected in intermittent-light flea traps were females, but by days 28-30, unfed males accounted for 78% of the population. This study demonstrated that afoxolaner chewable rapidly and effectively eliminated flea populations in infested dogs and homes.

Nexgard has also demonstrated high efficacy for multiple tick species including *Dermacentor variabilis*, *D. reticulatus*, *Ixodes ricinus* and *Rhipicephalus sanguineus* and *Amblyomma americanum*. It is a highly palatable beef flavored product that can be given with or without food. Adverse reactions are rare. In a U.S. field study, vomiting was seen in 17/415 (4.1%) cases administered afoxolaner.

Fluralaner, Bravecto®: Merck Animal Health

Fluralaner is also a member of isoxazoline class of insecticides that has shown potent acaricidal and insecticidal activity through a dual mechanism of binding to neuronal GABA- and glutamate-gated chloride channels in susceptible invertebrates. Numerous studies including a recent field study in dogs have shown that a single fluralaner dose administered orally as chewable tablet provides flea and tick control for twelve weeks. It is readily absorbed after single-dose oral administration, and has a long elimination half-life, long mean residence time, relatively high apparent volume of distribution, and low clearance. These pharmacokinetic characteristics help to explain the prolonged activity of fluralaner against fleas and ticks on dogs after a single oral dose. For best absorption, bioavailability and efficacy, it should be given with food. A more recent study measured the efficacy of fluralaner in dogs against adult *Ctenocephalides felis felis* and egg production. In the study, a single fluralaner flavored chew was administered at 25 mg/kg. On days -2, 28, 56, 84, 91, 98, 105, 112, and 120 post-treatment, each dog was infested with approximately 200 unfed cat fleas, *C. felis felis* (KS1 strain). Forty-eight hours after treatment and 48h after each infestation, eggs were collected over a 3-h period, counted and viability determined. Treatment of dogs with oral fluralaner provided a 100% reduction in flea counts 48h after treatment and within 48 h of every post-treatment infestation through day 122. Egg production from fluralaner treated dogs was reduced by 99.9% within 48 h after treatment and not a single egg (100% efficacy) was thereafter collected from treated dogs. Adult flea counts and egg production from the fluralaner-treated dogs were significantly lower than for
non-treated controls at all post-treatment evaluations ($P < 0.001$). This study showed that a single oral dose of fluralaner provided 100% efficacy against repeated flea infestations on dogs for 4 months. Fluralaner reduced egg production of female fleas by 99.9% and then killed every single female flea before any eggs could be produced following each subsequent re-infestation for the entire 122-day evaluation period.\textsuperscript{26}

It has proven efficacy against multiple tick species (Rhipicephalus sanguineus, Ixodes hexagonus, Ixodes ricinus, Dermacentor reticulatus and Amblyomma americanum).\textsuperscript{24}

Sarolaner, Simpria\textsuperscript{TM}: Zoetis Inc
Sarolaner is one of the newest insecticidal active ingredients from the chemical class of isoxazoline insecticides. It is scheduled for release in 2016 and has similar properties to other isoxazolines. More trials are currently being performed.

Flea and Tick Collars
There are a variety of flea and tick collars commercially available; the following are the collars that the author has used on a limited basis.

Deltamethrin 4%, Scalibor\textsuperscript{®}: Merck Animal Health
The author has used this collar mainly as a form of tick control but it has reasonably good flea control efficacy. It has deltamethrin formulated in a polymer matrix system that has been evaluated for the prevention of ticks and flea control on dogs.\textsuperscript{27,28}

Imidacloprid 10%/flumethrin 4.5%, Seresto\textsuperscript{®}: Bayer Animal Health
The efficacy of a slow-release insecticidal and repellent collar containing 10% imidacloprid and 4.5% flumethrin (Seresto) in preventing flea and tick infestation has been very effective in both dogs and cats. In one study, the imidacloprid/flumethrin collar proved to reduce tick counts by at least 90% and flea counts by at least 95% for a period of at least 7-8 months in cats and dogs under field conditions.\textsuperscript{29} In another study, 82 dogs were evaluated for flea and tick numbers after the imidacloprid/flumethrin collar was placed on them. 79/82 (96.3%) included dogs were initially heavily infested by ticks (46.9 +/- 65.7), and 53/77 (68.8%) were infested by 20 to 50 fleas. In addition, some of the flea-infested dogs (18.9%) had signs of flea allergic dermatitis (FAD). Two days after treatment, 49 (60.5%) and 9 (11.7%) dogs were still infested by live ticks and fleas, respectively. However, the mean intensity of ticks decreased to 3.5 (+/- 4.3) with a reduction of 92.5%. Except for sporadic cases, no attached ectoparasites were found on dogs from the day 14 visit until the end of the investigation. Cases of FAD resolved without any other treatment within 30 days.\textsuperscript{30}

References


TOPICAL THERAPY FOR CANINE ATOPIC DERMATITIS

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Overview of the Issue
Topical therapy can play an integral role in the management of the veterinary patient with canine atopic dermatitis. The overall pruritus in allergic dermatitis patients is often the result of a summation effect of various factors that has an individual animal exceeding its pruritic threshold. Secondary bacterial and yeast skin infections commonly occur and typically exacerbate pruritus in most dogs with allergic dermatoses. Dogs often experience exacerbations when an allergic trigger or secondary infection acts as a flare factor to place the animal above its pruritic threshold. Topical therapies can be utilized in the management of canine atopic dermatitis to try and increase the overall pruritic threshold in an allergic animal. They can at times function as a sole therapy but more commonly they play an important role when combined with other management therapies / strategies. The obvious advantage of any topical therapy for atopic dermatitis is any potential anti-pruritic effects it may have for the individual animal. This might be achieved if a topical therapy removes allergens from the skin surface, provides pharmacologic antipruritic effects, attempts to correct skin barrier dysfunction, and/or reduces surface colonization by yeast and bacteria.

Objectives of the Presentation
In this lecture a panel of speakers will share their views about what the roles topical therapy can play in the management of atopic dermatitis. It is beyond the scope of these notes to review every topical therapy product on the market; instead the focus will be on principles to consider efficacy of some specific topical therapy agents and the scientific evidence whenever possible to support use.

General considerations about topical therapy
When selecting a topical therapy product it is important to consider not only the desired therapeutic goal and consequently the most appropriate agent or active ingredients but also the type of formulation or delivery system. Formulation choice will depend on the amount of surface area requiring topical treatment, presence or absence of hair, type of lesion (deep lesions may be less impacted by topical therapy), amount of residual activity desired, practicality of vehicle application and acceptance of its use by pet or owner. The most commonly used formulation is a shampoo, as it can be used when large haired areas of the animal are requiring therapy. Other topical therapy formulations include “wipes” (towelettes/pledgets), sprays, “spot-ons,” solutions (soaks or rinses), lotions, gels, creams and ointments.

There are some advantages to the use of topical therapy versus systemic therapy. Topical therapy can provide direct delivery to the skin itself and typically would avoid systemic side effects. However, anything applied topically, particularly to already inflamed skin, has the
potential to cause irritation or contact dermatitis. Topical therapeutics can be absorbed systemically (either percutaneously or through oral ingestion from grooming behavior). This systemic absorption, if it occurs, may result in side effects, toxicities or drug interactions. If a patient’s condition worsens after initiation of a topical therapy, then it is important to discontinue the use of that product immediately and recommend that the owners rinse the patient in lukewarm water, in case any amount of the product remains on the skin. The dog should be re-examined in order to, if possible, determine the reason for the worsening in clinical signs, such as irritation, contact reaction/sensitivity, drug interaction, disease progression, or incorrect original diagnosis.

**Anti-Pruritic Effects of Bathing**

There is some evidence that either the mechanical action of shampoo therapy or the vehicles, regardless of the agent within a shampoo itself, can have a beneficial effect in the management of allergic pruritus. A double blinded, placebo controlled study to evaluate an antipruritic shampoo that contained lactoferrin, chlorhexidine, piroctone olamine, chitosan, essential fatty acids and glycerine found that both the test shampoo and placebo (shampoo vehicle) were equally effective at significantly reducing pruritus in dogs with mild to moderate allergic pruritus. This benefit from frequent (weekly) shampoo therapy regardless of the agent in the shampoo has been noted in another study and may be the result of the removal of scales/crusts or allergens from the skin surface or possibly the products used had a moisturizing effect.

There is a need for well designed, controlled studies with evidence to support the superiority of any of the agents used in shampoos with a claim to reducing pruritus. The published guidelines for the treatment of canine atopic dermatitis by the previous International Task Force on Canine Atopic Dermatitis and the most recent one by the International Committee on Allergic Diseases of Animals recognize that weekly bathing with a non-irritating shampoo and lukewarm water can provide a direct soothing effect to the skin, physically remove allergens and microbes and increase skin hydration. It is important to consider that any selected shampoo therapy must cause minimal disruption to the epidermal lipid barrier. The ideal shampoo for the management of an atopic dog should be gentle, lipid barrier sparing, and prevent surface bacterial and yeast overgrowth.

**Topical Pharmacological Agents with Anti-Pruritic Activity**

Anti-pruritic agents are most effective in formulations that permit some residual activity on the skin. There are some agents in topical shampoos and sprays that may provide very transient effects. Topical anesthetics such as pramoxine hydrochloride or lidocaine may provide brief, anti-pruritic effects but require frequent application. However, frequent application can result in an even more transient effect over time. Pramoxine is often used in combination with other agents such as colloidal oatmeal, hydrocortisone and menthol. Colloidal oatmeal is found in a number of products and can have a hygroscopic effect on the skin. Menthol creates a cooling sensation, substituting pruritus for a different sensation (cooling or tingling). Once again, there is little evidence thus far for the efficacy of these products but, they are safe and well tolerated although menthol has traditionally been avoided in cats due to adverse reactions.
The efficacy of topical glucocorticoids for the treatment of allergic pruritus in dogs has been confirmed in a number of randomized clinical trials\textsuperscript{6-8} and their use is recommended in the published Practice Guidelines for management of canine atopic dermatitis\textsuperscript{3,4}. There is strong evidence that the use of medium potency glucocorticoid sprays such as, low-dose (0.015\%) triamcinolone spray (Genesis\textsuperscript{TM} Spray, Virbac) or 0.0584\% hydrocortisone aceponate (Cortavance, Virbac, Carros, France) can be very effective for the short term management of allergic pruritus\textsuperscript{3,4,6-8}. The latter spray is not available in the United States but hydrocortisone aceponate is used commonly in many other countries and its unique metabolism occurs entirely within the skin, thus having little systemic absorption. Both of these topical glucocorticoid sprays are labeled for short term use only and the frequency and duration of their application should be monitored closely for adverse effects of topical glucocorticoids. The most common adverse effects of prolonged application of potent, topical glucocorticoids are cutaneous atrophy (skin thinning), superficial follicular cysts (milia) and comedones but can also include alopecia, secondary pyoderma and calcinosis cutis\textsuperscript{9}.

There are many other veterinary products on the market that contain topical glucocorticoids; although untested in randomized clinical trials they will likely provide clinical benefit for allergic dermatoses. They can be found in shampoos, leave on rinses, sprays, lotions, creams and ointments. Glucocorticoid containing lotions and creams are impractical for treating large areas but if pruritus is limited to small areas, such as feet or pinnae, they can be useful. The efficacy of these products and also their side effects will depend on the formulation, duration of application, and potency of the corticosteroid. It is important to be aware of the potency of the topical glucocorticoid in prescribed products since the more potent the topical glucocorticoids the greater the risk for adverse effects to occur. Topical glucocorticoids can also have systemic side effects of iatrogenic hypercortisolemia (iatrogenic Cushing’s disease) due to percutaneous absorption; this is of particular concern in small breed dogs.

Tacrolimus (Protopic\textsuperscript{R}, Astellas Pharma, Tokyo, Japan) is a topical macrolide that is a calcineurin inhibitor similar in activity to cyclosporine A. It is available as an ointment in 2 different strengths (0.1\% and 0.03\%). Tacrolimus (0.1\%) has been shown to be effective in the management of dogs with atopic dermatitis, especially if the affected body area is small\textsuperscript{10,11}. It is most efficacious when applied twice daily for a week and then tapered as needed to control clinical signs. The onset of action of tacrolimus is slow so, it is less helpful in treating acute flares of pruritus. In some patients it can be irritating and it may be cost prohibitive for some clients. There is a recent double blinded, randomized placebo controlled clinical trial that reported the clinical efficacy of a novel topical cyclosporine A (CsA) formulation for the treatment of atopic dermatitis in dogs\textsuperscript{12}. This new formulation uses nanocapsules of chitosan to protect the CsA from degradation and allow better penetration. To date this is just an experimental product.

**Addressing Skin Barrier Dysfunction in Allergic Dermatoses**

In humans with atopic dermatitis, skin barrier dysfunction has been recognized to be a key factor in the pathogenesis of atopic dermatitis. There is evidence that dogs with atopic dermatitis also have dysfunction of their skin barrier, however, whether this is a cause or effect of atopic dermatitis is debated. Nevertheless, a decrease in ceramides has been
identified in the skin of dogs with atopic dermatitis and demonstrated to result in increased trans-epidermal water loss \textsuperscript{13,14}. It is proposed that epidermal barrier dysfunction leads to increased allergen presentation to the immune system and this then results in sensitization and induction of the inflammatory cells and mediators that cause canine atopic dermatitis\textsuperscript{5,15}.

Therapies to repair barrier function are commonly used in human dermatology where emollients are used to decrease trans-epidermal water loss. Emollients work best when applied immediately after a bath. Examples of emollients are safflower oil, sesame oil, or lanolin. HyLyt EFA Bath oil\textsuperscript{®} (DVM Pharmaceuticals, Teva, Bayer) is a commercial veterinary emollient oil product. There are now veterinary lipid replacement products available as spot-ons or sprays. They contain essential fatty acids, essential oils and/or lipids (ceramides or phytosphingosine [pro-ceramide]). There have been limited clinical trials investigating the use of these products. Allerderm\textsuperscript{®} Spot-On (Virbac SA, Carros, France) is a topical spot-on lipid complex containing ceramides, cholesterol and free fatty acids that has been shown to help restore the ultrastructural lipid abnormalities in a small number of dogs with atopic dermatitis\textsuperscript{16}. In a clinical trial the same product was used in 8 dogs and a significant reduction in the dog’s Canine Atopic Dermatitis Extent and Severity Index (CADES-03) scores was seen after 6 weeks of biweekly applications\textsuperscript{17}. Another study had similar results using the topical Dermoscent Essential 6 spot on (Dermoscent\textsuperscript{®}, LDCA, France, Bayer Animal Health, USA) product that contains fatty acids and ‘essential oils’\textsuperscript{18}. Initial studies looking at topical products to address barrier dysfunction show promise but, well designed clinical trials are needed to prove the efficacy of these topical products in the management of atopic dermatitis.

**Role of Topical Antimicrobial Therapy in Atopic Dermatitis**

Topical antimicrobial therapy is useful in the management of allergic dermatoses to help treat or prevent secondary skin infections with bacteria and yeast. These secondary skin infections, when they occur, can contribute to the overall pruritus in allergic dermatoses. There are a variety of antimicrobial topical formulations and a large number of agents that can be found in various combinations. These include antibiotics, acetic/boric acid, benzoyl peroxide, chlorhexidine, ethyl lactate, fusidic acid, mupirocin, nisin, phytosphingosines, povidone iodine, silver sulfadiazine, topical azoles (miconazole, ketoconazole), and triclosan, and tris-EDTA.

Benzoyl peroxide, chlorhexidine and ethyl lactate are the three antimicrobial agents that have been the most extensively studied. Benzoyl peroxide oxidizes bacterial organisms resulting in its antibacterial activity. It also has degreasing and keratolytic properties and can cause excessive drying and possible irritation of the skin. Owners should be warned that it may bleach fabric and hair and to adhere to expiration dates, since it may degrade with time.

Chlorhexidine has its antibacterial effects by disrupting the bacterial cell wall and at higher concentrations causing precipitation of intra-cellular ATP. It also has antifungal properties at concentrations of 3 to 4%. It is not inhibited or inactivated by organic debris (crusts, scales, exudate). Chlorhexidine is available as a shampoo (varying concentrations and concomitant ingredients), surgical scrub, sprays, wipes and solutions (varying concentrations).
Chlorhexidine has the strongest scientific evidence supporting its efficacy in treating both bacterial and yeast skin infections\textsuperscript{19-21}. However, the most efficacious concentration for antibacterial activity differs amongst studies. Chlorhexidine should be above 3% or combined with miconazole to have efficacy against Malassezia. When combined, chlorhexidine and miconazole seem to be synergistic against both yeast and bacteria.

Ethyl lactate is broken down by bacterial lipases to lactic acid and ethanol, which both have antibacterial properties. Lactic acid results in lowering of the pH of the skin and ethanol can solubilize lipids. It is typically well tolerated, although studies to date are contradictory on its efficacy. An \textit{in vivo} study directly compared the efficacy of seven antimicrobial shampoos (active ingredients included ethyl lactate, benzoyl peroxide, chlorhexidine, and acetic/boric acid) found chlorhexidine containing products to be the most efficacious\textsuperscript{22}.

Antimicrobial shampoos are beneficial for use in dogs that develop recurrent surface bacterial overgrowth or bacterial and/or \textit{Malassezia} infection. These products offer simple gentle cleansing, remove infectious debris and kill surface bacteria and \textit{Malassezia}. Medicated antibacterial and antifungal “wipes” are applied to the skin once or twice daily and do not require rinsing afterwards. They are particularly useful in intertriginous areas, such as tail folds, facial folds, and perivulvar or interdigital regions. Topical 2% mupirocin ointment is a useful topical antibiotic for topical therapy of small discrete areas (individual small lesions or skin folds).

With the increasing prevalence of methicillin resistant \textit{Staphylococci} species as a cause of pyoderma in dogs with allergic dermatoses topical antimicrobial therapy is a critically important treatment modality to consider utilizing. There is increasing research being done to investigate antimicrobial therapies not previously in routine use in small animal dermatology. Such products include sodium hypochlorite (household bleach), hypochlorous acid, and lantibiotics such as nisin wipes\textsuperscript{23}.

Summary

The possible benefits of topical therapy that have been reviewed should always be considered in the management of atopic dermatitis. Ultimately it is owner compliance that determines the success of topical therapy. It is of no benefit to the patient if the client will not use the product or uses it improperly. It is important to provide clear, detailed, written (whenever possible) instructions as to how to apply the product, length of skin contact time and whether or not it is meant to be rinsed off. The temperament of the pet, the cost of the topical therapy, the owner’s own physical and time constraints, and the aesthetics of the topical product will all influence owner compliance.

References


DIAGNOSIS OF IMMUNE-MEDIATED DISEASE

Tim Nuttall

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1. Introduction

Immune-mediated diseases are uncommon, but a precise diagnosis helps us offer an accurate prognosis and select appropriate treatment. Most clinicians rely on biopsy and histopathology, but the results can be non-specific and/or misleading if inappropriate lesions are selected. The importance of understanding the signalment, taking a good history and performing a thorough clinical examination should not be underestimated. It’s also important to know when other tests are appropriate. Diagnostic steps include:

- Looking at the signalment and taking a good history
- Thorough clinical examination
- Cytology
- Biopsy and histopathology
- Immunohistochemistry
- Haematology, biochemistry, serology and urinalysis
- Anti-nuclear antibody tests

2. Signalment and history

Many immune-mediated diseases have distinct breed predispositions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Breed(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus foliaceus (PF)</td>
<td>Spaniels, Akita, chow</td>
</tr>
<tr>
<td>Insecticide-trigger contact PF (ITC-PF)</td>
<td>Large breed dogs (72% &gt;20kg)</td>
</tr>
<tr>
<td>Epidermolysis bullosa acquisita (EBA)</td>
<td>Great Dane</td>
</tr>
<tr>
<td>Mucous membrane pemphigoid (MMP)</td>
<td>German shepherd dog</td>
</tr>
<tr>
<td>Classical discoid lupus erythematosus (CDLE)</td>
<td>Shetland sheepdogs, rough collies, German shepherd dogs, Siberian huskies</td>
</tr>
<tr>
<td>Generalised DLE (GDLE)</td>
<td>Chinese crested dog?</td>
</tr>
<tr>
<td>Vescicular cutaneous lupus erythematosus (VCLE)</td>
<td>Rough collie, Border collie, Shetland sheepdog</td>
</tr>
<tr>
<td>Exfoliative CLE (ECLE)</td>
<td>German short haired pointer</td>
</tr>
<tr>
<td>Mucocutaneous lupus erythematosus (MCLE)</td>
<td>German shepherd dog</td>
</tr>
<tr>
<td>Systemic lupus erythematosus (SLE)</td>
<td>German shepherd dogs, rough collies, Shetland sheepdogs, beagles, Afghan hounds, old English sheepdogs, poodles, Irish setters</td>
</tr>
<tr>
<td>Alopecia areata (AA)</td>
<td>Dachshunds?</td>
</tr>
<tr>
<td>Mural isthmic folliculitis</td>
<td>Lundehunds</td>
</tr>
<tr>
<td>Anal furunculosis, metatarsal fistula and German shepherd dog pyoderma</td>
<td>German shepherd dog</td>
</tr>
<tr>
<td>Injection site vasculitis</td>
<td>Bichon frise, poodles, Yorkshire/silky terriers</td>
</tr>
<tr>
<td>Proliferative arteritis of the nasal philtrum</td>
<td>Saint Bernard</td>
</tr>
<tr>
<td>Cutaneous and renal glomerular vasculopathy (CRGV/Alabama rot)</td>
<td>Greyhound</td>
</tr>
<tr>
<td>Idiopathic ear margin vasculitis</td>
<td>Dachshund</td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>Rough collies, Shetland sheepdogs, beauceron</td>
</tr>
<tr>
<td>Familial cutaneous vasculopathy</td>
<td>German shepherd dog</td>
</tr>
<tr>
<td>Adverse drug reaction (sulphonamides)</td>
<td>Doberman</td>
</tr>
<tr>
<td>Uveodermatologic syndrome</td>
<td>Akita</td>
</tr>
</tbody>
</table>
Most conditions affect young adult to middle aged animals. They are rare in young animals except in some predisposed breed conditions (e.g. ECLE in German short haired pointers) and drug or vaccine induced conditions. ITC-PF affects older animals (median 9 years). Females are predisposed to CDLE and SLE, but there is otherwise little sex bias. It is important to look for systemic neoplasia or other conditions that may have triggered (or that may complicate treatment or worsen the prognosis) immune-mediated diseases in older animals.

The history should be carefully evaluated for potential triggers, which may include vaccines, drugs and (especially in lupus) UV exposure. Relevant drug exposure is usually within two weeks of the onset of clinical signs, and a drug reaction is unlikely with administration more than 6 weeks prior to the clinical signs. However, it is unknown how newer long-acting systemic drugs interact with the immune system. ITC-PF has been associated with metaflumizone/amitraz (Promeris Duo®), fipronil/s-methoprene/amitraz (Certifect®) and dinotefuran/pyriproxyfen/permethrin (Vectra 3D®). Sulphonamides are most commonly associated with adverse drug reactions (ADRs), although these have been reported with a very wide range of drugs. It can be helpful to use the Naranjo algorithm to determine whether the drug association is definite, probable, possible or doubtful. Immune-mediated ADRs can be drug-triggered (i.e. the drug triggers persistent disease in predisposed individuals) or drug-induced (i.e. the drug induces the condition which should quickly resolve on withdrawal of the drug).

3. Clinical examination
A. Understanding the significance of the clinical signs

<table>
<thead>
<tr>
<th>Clinical lesion</th>
<th>Significance</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crusts</td>
<td>Exudation, discharge, haemorrhage</td>
<td>Common and non-specific</td>
</tr>
<tr>
<td>Scale</td>
<td>Exfoliation; chronic disease</td>
<td>ECLE, PF, pemphigus vegetans (Pveg), sebaceous adenitis (SA), GDLE, ADRs</td>
</tr>
<tr>
<td>Lichenification</td>
<td>Chronic disease</td>
<td>GDLE</td>
</tr>
<tr>
<td>Erosions</td>
<td>Superficial disease above basement membrane; acute/subacute disease</td>
<td>PF, ECLE, CDLE, GDLE</td>
</tr>
<tr>
<td>Ulcers that move with the skin</td>
<td>Deep disease affects whole epidermis, basement membrane and upper dermis; acute/subacute disease</td>
<td>SLE, VCLE, CDLE, MCLE, MMP, EBA, pemphigus vulgaris (PV)</td>
</tr>
<tr>
<td>Skin moves over ulcers</td>
<td>Full thickness necrosis</td>
<td>Vasculitis, Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN); anal furunculosis, GSD pyoderma</td>
</tr>
<tr>
<td>Cutaneous atrophy and scarring</td>
<td>Vasculopathy and hypoxia</td>
<td>Vasculitis, SLE, dermatomyositis</td>
</tr>
<tr>
<td>Alopecia</td>
<td>Targets follicles</td>
<td>AA, pseudopelade, mural ishmic folliculitis, feline mural folliculitis (degenerative mucinotic or lymphocytic), dermatomyositis (+ other vasculopathies), SLE</td>
</tr>
<tr>
<td>Nail loss</td>
<td>Specific for symmetrical lupoid onychodystrophy (SLO); with other clinical signs in other conditions</td>
<td>SLO, vasculitis, DLE</td>
</tr>
<tr>
<td>Clinical lesion</td>
<td>Significance</td>
<td>Examples</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-------------------------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Draining sinus tracts</td>
<td>Lesions affect subcutaneous tissues</td>
<td>Vasculitis, panniculitis, SLE, anal furunculosis, GSD pyoderma</td>
</tr>
<tr>
<td>Mucosal</td>
<td>Mucosa; mucocutaneous junctions</td>
<td>MCLE, MMP, EBA, PV, SJS, TEN</td>
</tr>
<tr>
<td>Affects footpads</td>
<td></td>
<td>EBA, vasculitis</td>
</tr>
<tr>
<td>Target lesions</td>
<td></td>
<td>Erythema multiforme (EM)</td>
</tr>
<tr>
<td>Purpura, petechiae, ecchymoses</td>
<td>Vascular lesions</td>
<td>Vasculitis</td>
</tr>
<tr>
<td>Extremities</td>
<td></td>
<td>Vasculitis</td>
</tr>
<tr>
<td>Muscle atrophy</td>
<td></td>
<td>Dermatomyositis</td>
</tr>
<tr>
<td>Loss of pigment</td>
<td></td>
<td>Vitiligo, DLE, uveodermatological syndrome</td>
</tr>
<tr>
<td>Ocular signs</td>
<td></td>
<td>Uveodermatological syndrome</td>
</tr>
<tr>
<td>White limbs</td>
<td></td>
<td>Equine photoactivated vasculitis (pastern vasculitis)</td>
</tr>
</tbody>
</table>

Immune-mediated inflammation is often associated with hypopigmentation, possibly due to targeting of melanocytes and/or scarring. White hairs may regrow following AA. Reticulated hyperpigmentation is seen in GDLE. Peri-lesional hyperpigmentation also occurs in MCLE.

B. Localised ITC-PF
Dogs with localised ITC-PF present with pustules, erosions, alopecia and crusts typical of PF in areas associated with topical administration of the products (e.g. between the shoulder blades, dorsal neck, along the spine and running down the flanks).

C. Differentiating PF from pyoderma
Staphylococcal exfoliative toxins can cleave Dsg1 leading to loss of cellular adhesion and acantholysis. It is therefore important to carefully distinguish PF from acantholytic pyoderma. This may be difficult as the response to antibiotic treatment will not distinguish PF from an antibiotic-resistant infection (e.g. with MRSP), and while bacteria may be missed on cytology, histology and culture they may also be present in cases of PF with secondary infection. The history and clinical signs can help differentiate these conditions.

<table>
<thead>
<tr>
<th></th>
<th>PF</th>
<th>Pyoderma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symmetrical facial and periocular lesions</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Medial pinna affected</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Footpads affected</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Trunk initially affected</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Irregular, polycyclic or annular pustules</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Coalescing pustules</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Non-follicular pustules</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Flaccid pustules</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Turgid pustules</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Epidermal collarettes</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Expanding collarettes lesions</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Palisading crusts</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Moist erosions</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
D. Nikolskiy’s sign
Nikolskiy’s sign is the ability to split the epidermis forming an erosion or ulcer with digital or blunt force. There are a number of clinical variants.

<table>
<thead>
<tr>
<th>Nikolskiy’s sign</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>Normal skin splits distant from primary lesions</td>
<td>PV</td>
</tr>
<tr>
<td>Marginal</td>
<td>Normal peri-lesional skin splits</td>
<td>PV</td>
</tr>
<tr>
<td>Pseudo</td>
<td>Erythematous peri-lesional skin splits</td>
<td>EM/SJS; VCLE</td>
</tr>
</tbody>
</table>

E. Differentiating autoimmune subepidermal blistering diseases (AISBDs)
AISBDs are characterized by vesicles and ulcers associated with dermo-epidermal separation. Differentiating these from each other and similar conditions can be helpful in determining treatments and prognosis but the special techniques to identify the target antigens are not routinely available. Clinicians should, therefore, look at the breed, type and distribution of skin and mucosal lesions, depth of dermo-epidermal separation, and the inflammatory pattern to determine the most likely diagnosis. The lesions are often painful. Pruritus is less common but may be severe. Other problems can include lethargy, pyrexia, lymphadenopathy, anorexia and hypersalivation depending on the extent and severity of the skin and mucosal lesions.

<table>
<thead>
<tr>
<th>AISBD</th>
<th>Frequency</th>
<th>Breed</th>
<th>Clinical features</th>
<th>Predominant lesion distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP</td>
<td>50%</td>
<td>GSD</td>
<td>Heal by scarring; symmetrical lesions</td>
<td>Oral cavity; mucocutaneous junctions; pinnae</td>
</tr>
<tr>
<td>EBA</td>
<td>25%</td>
<td>Great Dane</td>
<td>Symmetrical lesions</td>
<td>Mucosal surfaces; mucocutaneous junctions; skin; pinnae; footpads;</td>
</tr>
<tr>
<td>Junctional EBA</td>
<td>Rare</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BP</td>
<td>10%</td>
<td></td>
<td></td>
<td>Skin (frictional sites)</td>
</tr>
<tr>
<td>Mixed AISBD</td>
<td>Rare</td>
<td></td>
<td></td>
<td>Oral cavity; mucocutaneous junctions</td>
</tr>
<tr>
<td>Linear IgA dermatosis</td>
<td>Rare</td>
<td></td>
<td></td>
<td>Oral cavity; footpads</td>
</tr>
<tr>
<td>Pemphigoid of gestation</td>
<td>Rare</td>
<td>Pregnancy</td>
<td>Oral cavity</td>
<td></td>
</tr>
<tr>
<td>Bullous SLE</td>
<td>Rare</td>
<td></td>
<td></td>
<td>Oral cavity; footpads; skin (frictional sites)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vesicle</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flaccid vesicle</td>
<td>PV; EM/SJS; VCLE</td>
</tr>
<tr>
<td>Turgid vesicle</td>
<td>AISBDs</td>
</tr>
<tr>
<td>Haemorrhagic vesicle</td>
<td>AISBDs</td>
</tr>
<tr>
<td>Erythematous margin</td>
<td>AISBDs; EM/SJS; VCLE</td>
</tr>
</tbody>
</table>

F. Erythema multiforme complex
The EM complex in dogs has been split into five groups - EM minor, EM major, SJS, SJS-TEN overlap syndrome and TEN. However, apoptosis is not a unique feature of EM. Histopathology does not reliably differentiate between the various forms, as there is extensive overlap in the changes between the different stages of disease and clinical manifestations. A final diagnosis relies on careful evaluation of the clinical signs and history.
G. SLE

SLE is a rare multi-systemic condition with variable clinical signs depending on the affected organs and tissues. Most cases develop lupus-specific cutaneous lesions, but non-lupus specific cutaneous lesions include panniculitis, vasculitis and type I-bullous SLE.

<table>
<thead>
<tr>
<th>Major signs</th>
<th>Minor signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-erosive polyarthritis</td>
<td>Pyrexia of unknown origin</td>
</tr>
<tr>
<td>Dermatological lesions</td>
<td>CNS signs, such as seizures</td>
</tr>
<tr>
<td>Coombs positive anemia</td>
<td>Pleuritis (non-infective)</td>
</tr>
<tr>
<td>Significant thrombocytopenia</td>
<td>Pericarditis (non-infective)</td>
</tr>
<tr>
<td>Glomerulonephritis (proteinuria)</td>
<td>Altered CD4⁺:CD8⁺ ratio</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>Polymyositis</td>
</tr>
<tr>
<td>Marked neutropenia</td>
<td>ANA positive</td>
</tr>
</tbody>
</table>

4. Cytology

Most immune-mediated diseases show non-specific cytology. However, cytology from pustules or fresh crusts will usually show neutrophils and acantholytic cells in pemphigus foliaceus. Cytology can also be helpful in identifying inflammatory nodular lesions, infectious diseases and/or secondary bacterial infections.

5. Biopsy and histopathology

Biopsy and histopathology is the most important way of confirming the diagnosis of an immune-mediated disease. Lesion selection is critical, as ulceration, necrosis, secondary infection and prior treatment can make the diagnosis difficult. Early lesions are most likely to yield a diagnosis, and the history and clinical signs should alert clinicians to the possibility of an immune-mediated disease and prompt biopsy. Early lesions include macules, papules, pustules, loss of pigment, haemorrhage, and intact nodules, vesicles and bullae. Punch biopsies are suitable for superficial and/or diffuse lesions. Deep wedge biopsies are better for lesions that involve the deep dermis or subcutaneous tissues. Elliptical wedges are also effective for spanning the margins of ulcers. P3 amputations are necessary for SLO. Most laboratories will examine at least three samples, so clinicians can collect several different lesions if unsure.
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6. Immunostaining

Immunohistochemistry or immunofluorescence can be used to detect the presence of antibodies, complement and cells targeting self-antigens. Briefly, direct immunostaining techniques employ specific regents directly on sections from the affected skin whereas indirect immunostaining involves incubating serum from affected animals with an appropriate substrate to demonstrate binding of circulating antibodies or other components. The sensitivity and specificity of the indirect approach relies on using appropriate and validated substrates (e.g. neonatal mouse skin, canine footpad, salt-split canine skin etc.) and reagents. These techniques were restricted to specialised centres and limited to IgG, IgM, IgA or complement factor C3. However, the advent of automated staining, immunoperoxidase labelling and much wider panels of specific antisera have made these techniques rapid, affordable and accurate even in formalin-fixed samples. Immunogold techniques (with gold particles coupled to the antibody) are similarly used for transmission electron microscopy. Immunostaining should not be used to replace conventional histopathology, but it can be useful to confirm the diagnosis in equivocal cases and/or where differentiating conditions is impossible with histopathology (e.g. in AISBDs). For example, 80% of sera from dogs with PF have positive indirect immunostaining with intercellular, top-heavy, web-like patterns most common. IgG4 antibodies are most common in the PF sera. With direct staining ~50% of biopsies from animals with PF show deposition of immunoglobulins or complement
between keratinocytes, and biopsies from animals with lupus show deposits at the basement membrane zone (lupus band). AISBDs usually show linear deposition of immunoglobulins along the basement membrane. Natural clefts or salt-split skin can be used to show the depth of separation. In EBA IgG binds the superficial dermis in clefts and to the dermal side of salt-split skin. In MMP and the other rare AISBDs the immunostaining is below the cleft.

However, false positives can be seen in chronic inflammation with leaking of serum immunoglobulin into the epidermis and other tissues (especially in nasal and footpad biopsies).

7. Serology and other tests
Knowing the major target antigens will allow the development of ELISAs to help diagnose some conditions. For example, IgG serology for Dsc1 is consistently positive in dogs with PF and negative in dogs with exfoliative staphylococcal pyoderma, and there is a recently developed canine Dsg3 ELISA to help in the diagnosis of canine PV. Titres may correlate with clinical severity and could, in theory, be used to monitor disease progression.

Antinuclear antibodies (ANA) target cell nucleus components. ANA tests involve incubating serum on nucleated cells, such as liver or cell lines. In addition to the titre, there are four patterns of staining (nuclear rim, homogenous, speckled or nucleolar). These have clinical relevance in humans, but this has not been proven in animals. A diagnosis of SLE requires a significant serum ANA titre. However, ANA may be present in up to 10% of clinically normal animals and are seen in other inflammatory conditions, infections and with some drugs. False negative results may occur due to technical reasons or prior treatment (e.g. glucocorticoids).

Coombs tests can be used to confirm the presence of surface-bound antibody or complement in animals with anaemia, although this doesn’t distinguish between primary autoimmune haemolytic anaemia (AIHA) and secondary immune-mediated haemolytic anaemia (IMHA). Rheumatoid factor (RF) assays may be used on serum and/or synovial fluid samples in animals with immune-mediated polyarthritis. However, low RF titres are common in other chronic inflammatory, infectious or neoplastic diseases.

Haematology, biochemistry, urinalysis and other tests may be appropriate in multi-systemic disorders. Diagnostic imaging may detect internal neoplasia and blood cultures may be necessary if sepsis is suspected. In appropriate cases and areas, tests for viruses (e.g. FIV, FeLV and FIP in cats, EVA and EIA), rickettsias (e.g. Rocky Mountain spotted fever, erlichiosis, borreliosis etc.) or Leishmania by serology or PCR should be considered.

8. Acknowledgements
These notes could not have been written without the excellent mentoring, support and other help from Thierry Olivry, Petra Bizikova, David Shearer and Michael Day.
Selected References


IMMUNE-MEDIATED DISEASES: TREATMENT OPTIONS

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1. Introduction
There are four important questions to be considered before embarking on the treatment of an immune-mediated disease, namely:

A. *Is the diagnosis correct?* You are embarking on a course of treatment that may be life-long and one that may have significant and even life-threatening side-effects. So you must be certain that you are dealing with an immune-mediated disease and one that has been as precisely defined as possible.

B. *The possible role of an adverse drug reaction (ADR).* ADRs can mimic any of the immune-mediated diseases. Is there a history of any parenteral or topical drug administration?

C. *Does the patient have any underlying disease that might make it more susceptible to treatment side-effects?* Therapy may be quite challenging for the body systems, and could have life-threatening side-effects in any but the fittest of patients.

D. *Do you have the right client?* The client must be patient and willing to work with you bearing in mind that treatment may be lifelong.

2. Drugs used for the treatment of immune-mediated skin diseases – in addition to corticosteroids

A. *Azathioprine.* This inhibits purine metabolism thus interfering with DNA and RNA synthesis and acts on rapidly dividing cells. It is metabolized to 6-mercaptopurine, which is active, and thence by xanthine oxidase and thiopurine methyltransferase to inactive metabolites. The fact that the cat has lower levels of the latter enzyme makes it much more toxic for this species. Azathioprine is a potent bone marrow suppressant, and patients should be monitored closely for all haematological parameters. It may also cause gastrointestinal side effects and liver damage with elevated alkaline phosphatase. It has been reported to cause pancreatitis, but affected dogs were also receiving corticosteroids. Complete blood counts and chemistry screens should be undertaken initially every 2 weeks, and then monthly to every three months. Doses are around 1.5-2.5 mg/kg.

B. *Cyclophosphamide.* This is a nitrogen mustard alkylating agent to which lymphocytes are highly susceptible, and especially B cells. It thus quickly reduces antibody levels. In addition to bone marrow suppression, it may cause a haemorrhagic cystitis even associated with granulomatous proliferation clinically mimicking neoplasia. Doses are
around 0.1-0.2 mg/kg daily or on alternate days, and its use is generally restricted to the induction phase of treatment after which it is discontinued. Haematology and blood chemistries should be undertaken every 2 weeks.

C. Chlorambucil. This is a useful alternative to cyclophosphamide which lacks the side effects on the bladder. Dose is 0.1-0.2 mg/kg every 24-48 hrs.

D. Mycophenolate mofetil. This drug also inhibits both B and T cells which has been evaluated in dogs at doses ranging from 2.0 mg/kg daily to up to 13 mg/kg TID. Side effects include bone marrow suppression, gastrointestinal abnormalities and an increased incidence of infections.

E. Ciclosporin. Well known for its use in atopic dermatitis, ciclosporin has been evaluated in some of the immune-mediated diseases. It inhibits lymphocyte function – especially that of T cells, and may help to normalise the Th1/Th2 imbalance in atopic dermatitis. It does not appear to be very effective as a monotherapy in immune-mediated diseases but may have a steroid-sparing effect.

F. Tacrolimus. This is a calcineurin inhibitor with a similar action to ciclosporin inhibiting T cell function. It is approved for the treatment of atopic dermatitis in man, and although its efficacy in the canine disease is unclear, it does have a role in the therapy of localized immune-mediated diseases when used at 0.1%.

G. Tetracycline and niacinamide. The mode of action of this combination is unknown, but there are favourable reports of its use in human medicine. Doses for dogs are 500 mg of each TID for dogs weighing > 10kg, decreasing to BID and then SID if there is a response. Doxycycline may be substituted for tetracycline at a lower dose of 5mg/kg. The author has had little success with this combination, except for mildly affected animals.

3. Pemphigus foliaceus (PF)
   A. Dogs. There are two phases in the treatment, namely remission induction and maintenance – and for both phases corticosteroids are the cornerstone of the therapeutic approach.
      a. Induction: For induction, prednisone or prednisolone are used at doses of 2.0 mg/kg to up to 6.0 mg/kg daily or divided BID. Some clinicians prefer the use of methylprednisolone at equivalent doses which may have less in the way of mineralocorticoid side-effects. Alternatively, triamcinolone may be employed at doses of 0.2-0.6 mg/kg, although there is limited availability of this drug in some countries.
      b. Maintenance: Such high doses are incompatible with life if used long-term, and the goal should be to gradually taper the dose after 3-5 weeks, or when good control is effected and move to a maintenance regimen using an alternate day dosage of around 1.0 mg/kg of prednisone or prednisolone or 0.1-0.2 mg/kg in the case of triamcinolone.
c. Concomitant immunosuppressive therapy. Approximately one third of cases will achieve remission using corticosteroids alone. In cases where there is a slow or incomplete response, azathioprine may be added at doses of 2.0-2.5 mg/kg daily – indeed some clinicians prefer to use combination therapy at the outset. If combination therapy is employed, the goal is again to achieve an alternate day regimen for maintenance whereby the corticosteroid is given on one day and the azathioprine on the other. Some have also recommended the concomitant use of the immunomodulating combination of tetracycline and niacinamide, but there is no good data supporting the efficacy of these drugs in PF. Topical corticosteroid therapy with an agent such as hydrocortisone aceponate (Cortevance®, Virbac) can be employed as adjunctive therapy on stubborn localized lesions, as can topical 0.1% tacrolimus.

d. Alternative corticosteroid regimens. A recent publication compared the use of standard corticosteroid therapy as noted above with one or more pulses doses of 10 mg/kg of prednisone for three days then reverting to a lower dose of 1-2 mg/kg for the next 4 days. This may be repeated for up to four times. The concomitant therapy employed was not identical between groups and so definitive conclusions are difficult. But it did appear to give at least equal if not superior results.

e. Supportive therapy. There are conflicting views on the advisability of concomitant broad spectrum antibiotics, with one study apparently showing no benefit. If cytology reveals invasion of the lesions with cocci, then antibiotic treatment would seem to be prudent. Warm water soaks can be helpful in removing crusts.

f. Prognosis. There are differing reports on the long-term prognosis, probably reflecting case selection, the rapidity with which the diagnosis is made and case management expertise. Overall, one can expect that some 75% of cases will achieve a good outcome with near-complete or total remission and a good quality of life. In well-controlled cases it may be worth tapering and withdrawing therapy to ascertain whether remission can be maintained without continued treatment, which has been recorded although unusual.

B. Cats. Although not as common as in dogs, PF is not infrequently diagnosed and carries a relatively good prognosis. Although it may be tempting to employ injections of long-acting methylprednisolone acetate (Depo Medrol®, Zoetis), the gradual loss of anti-inflammatory action towards the end of each dose makes control very difficult with this drug. Oral induction doses of 4-5 mg/kg of prednisolone or alternatively (and preferably) 0.6-2.0 mg/kg of triamcinolone together with 0.1-0.2 mg/kg of chlorambucil may be employed. Unfortunately, the severe bone marrow suppression that often results from the use of azathioprine in the cat precludes the use of this drug. The prognosis is quite good with careful management. In one case series of 44 cats followed for periods of 1-54 months the outcomes were generally favourable with only 4/44 dying from treatment-related causes during this time.
4. Other pemphigus variants
   A. *Pemphigus vulgaris*. This is an uncommon, but severe disease, and animals are usually systemically ill. Aggressive treatment with corticosteroids and azathioprine or chlorambucil is required, and support with broad spectrum antibiotics is essential. If remission is achieved, then tapering to maintenance is undertaken as described for PF. Prognosis is guarded with few animals surviving > 12 months. One possible case has been reported in a cat which was fatal.

   B. *Pemphigus erythematosus*. This is a cross-over between PF and lupus, with features of both. A relatively benign disease, it is usually responsive to tetracycline and niacinamide, or corticosteroids. If there is minor involvement, topical corticosteroids or tacrolimus may be employed. Avoidance of sunlight is an important aid.

   C. *Pemphigus-like drug eruptions*. These have been most commonly reported following the use of topically applied parasiticides including metaflumizone-amitraz (Promeris®, Zoetis, now discontinued), fipronil-amitraz-S-methoprene (Certifect®, Merial) and apparently less commonly dinotefuran/pyriprozifen/permethrin (Vectra 3D®, Ceva). But any drug, and any topical has the potential to initiate an ADR. The diagnosis is usually presumptive, as it is most unwise to confirm by drug challenge as a more severe and even fatal reaction may occur. Most cases will resolve upon discontinuing the drug, but in severely affected cases corticosteroids +/- immunosuppressive therapy may be needed for a period. Remission is the normal expectation.

   D. *Paraneoplastic pemphigus and pemphigus vegetans*. These are sufficiently rare that treatment guidelines cannot be given. The former carries a very poor prognosis due to the presence of underlying neoplasia. Immunosuppressive therapy should be attempted for the latter

5. Autoimmune subepidermal blistering diseases
   A. *Bullous pemphigoid*. Again, generally a severe disease with acute onset, although some may present with a gradual onset and a more benign mucosal pemphigoid is also reported. Prognosis is highly variable, with some cases apparently going into spontaneous remission. Combination therapy with corticosteroids and azathioprine is advised for acute cases, and broad spectrum antibiotics should be given. The more chronic and mild cases are controlled with corticosteroids alone or even with tetracycline and niacinamide. It is very rare in cats, and may respond to corticosteroids.

   B. *Epidermolysis bulla acquisita*. An acute onset disease usually with footpad involvement and the patients are systemically ill. Aggressive combination immunosuppressive therapy with azathioprine and corticosteroids together with supportive broad spectrum antibiotics are required. Warm water soaks are beneficial. The mortality rate is some 50%, but if well managed, cases can be maintained for a normal lifespan.
6. The lupus family

Many controversies surround these diseases, and the clinical spectra that should be included, and recent careful analysis of clinicopathological data is helping to better define the conditions, which can be broadly categorized into systemic lupus erythematosus (SLE) and cutaneous lupus erythematosus (CLE).

A. Systemic lupus erythematosus

a. Canine SLE. In this multisystem disease, which can have an acute or chronic onset, therapy is tailored to the organ system involved. All lupus is potentially exacerbated by sunlight, and so sun avoidance forms part of the therapy. Corticosteroids are generally employed, and may be all that is required in mild disease. However all cases should be carefully monitored for signs of other organ involvement. If there is autoimmune haemolytic anaemia, cyclophosphamide (initially intravenously and then orally) could be indicated, as could vincristine in cases exhibiting immune-mediated thrombocytopenia. Both should be used only for remission induction before moving on to corticosteroids alone for maintenance. Plasmapheresis has been employed for refractory cases. There have been impressive reports from France on the combined use of levamisole and prednisolone.\(^5\) This might seem paradoxical in the levamisole is an immune stimulant, and prednisolone has the reverse effects. The combination was more effective than was prednisolone alone and induced prolonged remission in \(>50\%\) of cases. However, in general the course is unpredictable and the long-term outlook guarded, although some cases can be maintained on low doses corticosteroids for long periods with a good quality of life.

b. Feline SLE. Reports of long-term management are few, but again, corticosteroids are employed supplemented if necessary by cyclophosphamide or chlorambucil.

B. Cutaneous lupus erythematosus

c. Discoid lupus (DLE). This usually presents with nasal depigmentation leading to erythema and later to ulceration. Avoidance of sunlight is important, and although some have advocated using sunscreens, the patient will usually lick them off rendering them ineffective. Tattooing was once advocated, but the ink ends up in the dermis where it is not effective. Tetracycline and niacinamide is reportedly effective in some 50-70\% of cases, but is slow to act and improvement may not be seen before two months. Use of high dose glucocorticoids (e.g. 2-4 mg/kg of prednisolone) followed by low doses or tetracycline/niacinamide for maintenance may give satisfactory results. However topical 0.1\% tacrolimus has emerged recently as the probably the treatment of choice. In chronic unresponsive cases, rotational skin flaps can be employed. Cases are sometimes reported that fulfil the diagnostic criteria that show more generalized disease for which corticosteroids probably represent the best approach.

d. Mucocutaneous lupus erythematosus. A variety of drugs have been employed in treating this condition that is seen most commonly in German shepherds, but
glucocorticoids seem to be the most effective at doses initially of 1-2mg/kg of prednisolone, and then tapering to alternate days. Some 10-20% of cases may go into remission and allow discontinuation of treatment without subsequent relapse. Concomitant antibiotics are not required. 

e. Vesicular cutaneous lupus erythematosus. This condition is seen most often in Shetland sheepdogs and rough collies and generally responds to high doses of corticosteroids followed by tapering for maintenance. However some may be slow to respond and require concomitant azathioprine. Alternatively, ciclosporin was effective in one case report, and in another a combination of tetracycline/niacinamide and topical tacrolimus was used with success. In view of the extensive ulceration, broad spectrum antibiotics should be employed until healing is complete. Again, some 20% of cases do not relapse upon discontinuation of therapy.

f. Exfoliative cutaneous lupus erythematosus of Germen short-haired pointers. This is a frustrating disease to manage. Although the skin disease is in no way life-threatening, lymphadenopathy and apparent joint pain suggests that there may be systemic complications. None of the interventions, which have included corticosteroids, ciclosporin, hydroxychloroquine and a TNFα antagonist, have induced remission, although some control was achieved with the former.

7. Vasculitis
This may occur in SLE, or more frequently in drug eruptions (including vaccines – especially rabies) or as a complication of bacterial, protozoal and viral diseases. But probably the majority of cases are the result of drug eruptions - including the administration of vaccines – notably rabies. Thus a careful search must be made for an underlying cause but in a proportion of cases none can be identified and the condition is idiopathic. In addition to managing any underlying cause, treatment generally involves the use of pentoxifylline at 10mg/kg TID (though there are anecdotal reports of pentoxifylline doses up to 20-30mg/kg PO TID), with the addition of corticosteroids and/or azathioprine in severe cases. There are also reports of the use of sulfasalazine (20-40 mg/kg TID) in idiopathic vasculitis. Approximately one third of cases will achieve remission using corticosteroids alone.

8. Final thoughts
Always be sure that the treatment is not worse than the disease. Many patients can be maintained comfortably with minimal disease by low doses of immunosuppressive therapy, whereas insisting on a total cure by raising the dosages might result in a bad outcome.

Selected References
CLINICAL APPROACH TO NON-INFLAMMATORY ALOPECIA

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Introduction
Causes of alopecia are numerous in dogs and include infections (e.g., dermatophytes, demodicosis, bacterial folliculitis, leishmaniasis), self-inflicted hair loss (from hypersensitivities or parasitism), immune-mediated diseases (sebaceous adenitis, dermatomyositis, alopecia areata), endocrinopathies, follicular dysplasias, etc.

Hair growth is influenced, among other things, by gonadal, adrenal, thyroid, pituitary and pineal hormones. Excesses, deficiencies and hormonal imbalances have been incriminated in a myriad of clinical syndromes in dogs. In some endocrinopathies (e.g., hypothyroidism, hyperadrenocorticism, hyperoestrogenism and pituitary dwarfism) the hormonal implication is well understood and these disorders are relatively well characterized clinically. However, other alopecic disorders may resemble endocrinopathies clinically (e.g., canine recurrent flank alopecia, alopecia-X, color dilution alopecia and other follicular dysplasias) and, in many instances, the final diagnosis can be more difficult to establish. The aim of these notes is to provide the clinician with a methodical clinical approach to canine alopecia, especially non-pruritic, non-inflammatory, symmetrical hair loss.

Signalment and history
When presented with a dog with alopecia, a complete history and physical examination should be taken in order to detect if abnormalities present in other organs. A history of polyuria-polydipsia, the presence of a pendulous abdomen or abnormal genitalia (testicular asymmetry or cryptorchidism, vulvar enlargement) may greatly influence further tests to be carried out. The history and the dermatological examination should allow the clinician to rule in/out the presence of pruritus. If significant, it should be investigated first. If pruritus is absent or minimal, one should determine whether the pattern of hair loss is focal, or symmetric and diffuse. In addition, one should look for presence of inflammation and/or any primary lesions such as papules and pustules. Skin scrapings, skin cytology and/or dermatophyte cultures are often indicated if such skin lesions are present. If pruritus, inflammation or any other primary lesions are absent, the next most pertinent diagnostic procedure to perform will be influenced by age of onset, breed and sexual status of the dog.

Age and timing at onset. The onset of alopecia should always be related to the dog’s age and any physiological and/or pathological event, management change or treatment. Alopecia sometimes occurs a few weeks after physiological events, such as pregnancy and lactation, or pathological events, such as severe systemic disease, shock or surgery (e.g., telogen defluxion). Failure of hair regrowth after clipping is suggestive of hypothyroidism, hyperadrenocorticism and alopecia-X. In Nordic breeds it is a common finding because hair follicle cycle in these breeds is longer than in other breeds.
Many disorders have an age at onset that is quite predictable. Congenital alopecia is present at birth; canine pattern alopecia often develops between 6 and 12 months of age; and demodicosis usually occurs before 1 year of age. Clinical signs due to hypothyroidism typically develop between 3 to 6 years of age, and spontaneous hyperadrenocorticism occurs generally in middle-aged to old dogs.

**Breed.** Some breeds are predisposed to alopecic conditions such as alopecia-X (e.g., Pomeranian, Keeshond, Malamute, miniature poodle), canine pattern alopecia (e.g., Dachshund, Chihuahua) and canine recurrent flank alopecia (e.g., boxer, Airedale).

**Coat color.** Coat color may provide useful diagnostic information in color-linked alopecia such as black hair follicular dysplasia, color dilution alopecia, and follicular lipidosis.

**Sexual status.** Hyperoestrogenism due to Sertoli’s-cell tumors and ovarian cysts or tumors may lead to alopecia. Prolonged estrus can be seen in bitches with hyperestrogenism whereas anestrus may be seen in bitches with hyperadrenocorticism and hypothyroidism.

**Spontaneous remission.** Spontaneous remission can occur in canine recurrent flank alopecia, anagen and telogen defluxions, and post-clipping alopecia not secondary to endocrinopathies. Alopecia areata may also resolve spontaneously and is often associated with regrowth of white hair (leucotrichia). Spontaneous remission is also seen in localized demodicosis and dermatophytosis; however, skin inflammation and scaling is usually observed.

**Progression.** Slow progression of alopecia is more indicative of a systemic problem (e.g., endocrinopathies). Seasonality is more indicative of canine recurrent flank alopecia or flea allergy dermatitis.

**Signs of internal disease.** Owners of dogs with hyperadrenocorticism often report polyuria, polydipsia and polyphagia. In canine hypothyroidism, the owner may describe signs that reflect the slowing of metabolism, such as lethargy, cold intolerance and weight gain.

**Previous treatments.** Endogenous and exogenous corticosteroids are notorious in causing Cushing's syndrome. One should not underestimate the effect of corticosteroids on hair growth (even when only applied topically such as in eyes), and on alkaline phosphatase and thyroid hormone levels. Focal alopecia may develop at site of injection, especially rabies vaccines.

**Physical examination**

Pendulous abdomen and hepatomegaly is frequently seen in hyperadrenocorticism. Enlarged lymph nodes can be seen in leishmaniasis. Abnormal genitalia (e.g., gynecomastia, testicular asymmetry or cryptorchidism) can be observed in hyperoestrogenism.

**Dermatologic examination**

Alopecia may be a feature of a myriad of skin diseases; therefore, thorough clinical examination of hair coat and skin is important. Presence of primary or secondary skin lesions (e.g., papules, pustules, scaling, crusts), follicular casts (e.g., in demodicosis and sebaceous
adenitis), skin thickness, aspect of hair shafts (broken or not) are some of the findings that can be very helpful to orient toward more specific diagnoses.

Erythema, papules, pustules, lichenification, self-trauma (recognized by broken hairs and excoriations) are all suggestive of an inflammatory process and pruritus. Thinning of the skin with prominent subcutaneous vessels and calcinosis cutis are pathognomonic of hyperadrenocorticism, whereas hypothyroidism is often accompanied by thickened and hyperpigmented skin without inflammation, unless a secondary bacterial infection is present. In canine recurrent flank alopecia, the alopecic areas are typically well demarcated and hyperpigmented.

Coat and skin color change. Coat color change (e.g., brown discoloration), especially noticeable in white dogs, is suggestive of licking. A regrowth of white hair, where the hair coat was formerly pigmented, is suggestive of alopecia areata.

Intense skin hyperpigmentation of alopecic areas is most commonly seen in dogs with recurrent flank alopecia and alopecia X. However, hyperpigmentation is also often observed in response to skin inflammation.

Pattern of alopecia. The pattern of the hair loss (e.g., focal, multifocal, moth-eaten, asymmetric, symmetric and diffuse) should be documented. Infectious alopecias generally develop a more asymmetric, multifocal, sometimes moth-eaten pattern, whereas endocrine alopecias and other hair cycle abnormalities are more symmetric in pattern. Allergies, however, may also present with bilaterally symmetric alopecia but the history will reveal that the dog demonstrates pruritus toward the affected areas.

Canine pattern alopecia (CPA), as the appellation implies, follows a predetermined distribution, and usually start around 6 months of age. In CPA ventral type, the alopecia develops along the ventral neck, chest and abdomen, the caudomedial aspect of thighs, perineum, and the base of the convex aspect of the pinnae and the temples. In CPA pinnal type, the alopecia involves the entire convex aspect of the pinnae.

Further investigation
If the results of the above evaluation have failed to produce a definitive diagnosis, further investigation is necessary. The diagnostic procedures should be selected according to the index of suspicion. Microscopic examination of skin scrapings, hair plucks (trichoscopy), Wood’s lamp examination (+/- fungal culture) and cytological evaluation of impression smears or swabs may all be required if primary or secondary lesions such as papules, pustules, erythema, scaling, crusting, follicular casts are seen.

Hematology, biochemistry and urine analysis are useful to evaluate the general health status of adult dogs with a non-inflammatory alopecic condition or if a systemic disease, which may lead to alopecia, is suspected. Hormonal tests (e.g., thyroid hormone profile, ACTH stimulation test, low dose dexamethasone suppression test) should be carried out if the clinical signs and results of blood or urinalysis suggest an endocrinopathy. In contrast, skin
biopsies may be the initial and unique diagnostic procedure performed if sebaceous adenitis is strongly suspected.

Selected References
Figure 1. Canine Alopecia

Alopecic breeds
- Chinese crested dog
- Mexican hairless dog
- Peruvian hairless dog
X-linked ectodermal dysplasia
Congenital hypotrichosis/alopecia

Hypersensitivities
- Atopic dermatitis
- Food hypersensitivity
- Flea bite hypersensitivity
- Parasites (e.g. Sarcoptes)

Congenital?

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<table>
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Cytology, skin scrapings, trichography, skin biopsy

Infections
- Staphylococci
- Demodex
- Dermatophytes
- Malassezia
- Leishmania

Endocrinopathies
- Hypothyroidism
- Hyperadrenocorticism
- Hyperoestrogenism
- Pituitary dwarfism

Immunemediated (microscopic inflammation)
- Sebaceous adenitis
- Dermatomyositis
- Post-injection alopecia (rabies vaccine)
- Adult-onset generalised ischemic dermatopathy (rabies vaccine-induced or idiopathic)
- Vasculitis
- Alopecia areata (pelade)
- Pseudopelade
- Eosinophilic mucinotic mural folliculitis
- Granulomatous degenerative mural folliculitis

Follicular dysplasias

Coat color-linked?

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- Recurrent flank alopecia
- Breed associated follicular dysplasias
- Portuguese Water dog
- Irish Water spaniel
- Chesapeake Bay retriever
- Pont Audemer spaniel

Miscellaneous
- Telogen defluxion
- Anagen defluxion
- Pattern alopecia
- Pinnal type
- Ventral type
- Neoplastic alopecia (e.g.)

Adapted from Manon Paradis, Clinical Veterinary Advisor 3rd ed. E. Coté ed. 2014
CHALLENGING ALOPECIA CASES: INTERACTIVE SESSION

Manon Paradis
Department of clinical sciences, Faculté de Médecine Vétérinaire,
Université de Montréal, St-Hyacinthe, Québec, Canada

Rosario Cerundulo
Veterinary Specialist Centre, Six Mile Bottom, United Kingdom

There are no written proceedings for this session — please use this space for your own notes.
DIAGNOSIS OF CANINE ATOPIC DERMATITIS (INCLUDING FOOD ALLERGY)

Claude Favrot

Dermatology Unit, Clinic For Small Animal Internal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Introduction
Canine Atopic Dermatitis (CAD) is the most frequent canine allergic dermatosis. It has been defined as a “genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens”. The revised nomenclature for veterinary allergy also takes into account dogs with clinical signs of atopic dermatitis but no demonstrable allergen-specific IgE: the term atopic-like dermatitis (ALD) was coined to describe this group of dogs.

In veterinary dermatology, cutaneous adverse food reaction (CAFR) and CAD have been historically considered as two different conditions. In fact, CAFR includes both immune-mediated and non-immune-mediated food intolerances and may be associated with a wide range of clinical signs such as gastrointestinal disturbances, urticaria, angioedema, and signs mimicking those of atopic dermatitis. This latter point has led the International Task Force on Canine Atopic Dermatitis to suggest that some cases of CAFR may trigger flares of atopic dermatitis. The clinical signs of CAD may thus be associated with sensitization to environmental allergens (CAD sensu stricto), food allergens (CAFR with clinical signs of CAD; Food-Induced Atopic Dermatitis [FIAD]) or with ALD. This presentation will describe the clinical features and diagnostic methods for dogs affected by CAD from whatever cause.

Clinical Signs of CAD
Signalment
The definition of CAD suggests strong breed and/or familial predispositions. Reliable evaluation of breed predispositions for veterinary diseases is complicated by the fact that the population at risk is often unknown. Several studies have addressed the question of breed predisposition for CAD. Some studies only mentioned the most frequently represented breeds, while others have been based on a comparison between atopic dogs and the hospital or insurance population. The former do not present any statistical analysis and the latter may be biased by the absence or the underrepresentation of healthy dogs. One single study is based on a large population of insured dogs but contains another potential bias — the authors did not make the diagnosis of CAD themselves but referred to the diagnosis by general practitioners, who may have used variable inclusion criteria. To further complicate the analysis, predisposed breeds may vary by geographical areas. One study is based on the comparison of a population of atopic dog and a validated population of healthy dogs. However, this study was limited to Switzerland. Some breeds such as West Highland white terriers, boxers and bulldogs are mentioned in virtually all these studies. Other breeds such as German shepherd dogs, golden retrievers or Labrador retrievers seem to be predisposed for CAD only in some geographical regions.
Reports of sex predisposition in CAD dogs are inconsistent. Some studies reported predisposition for male dogs, female dogs or for neither sex. We have recently studied a population of 843 dogs with CAD and no sex predilection was detected. However, sex predispositions were detected in some breeds such as golden or Labrador retrievers (more female) or boxer (more male) dogs.

The typical age at onset of CAD is reported to be between 6 months and 3 years. We have recently shown that 78% of dogs with CAD present with clinical signs before three years of age. This means that some dogs with CAD develop initial clinical signs later in life.

History
Information regarding the history of the affected dog should be recorded carefully. Some important questions have already been mentioned (age at onset, breed, familial predisposition) but other important questions include: seasonality, presence of pruritus *sine materia* at onset (pruritus not associated with lesions or another dermatosis) and efficacy of previous treatment.

Clinical signs of CAD may or may not be seasonal but seasonality is often present at onset (42-75%). Approximately 80% of dogs with seasonal signs are symptomatic in spring or summer while the others exhibit signs in winter or autumn. Some dogs with non-seasonal disease exhibit worsening of clinical signs during one specific season.

Pruritus must be present and its absence rules out the diagnosis of CAD. In fact, some dogs with CAD exhibit pruritus initially with no skin changes. This feature was recorded in 61% of affected dogs in our recent study. As well, 43% of dogs with CAD presented first with an episode of otitis externa. CAD dogs are often treated with glucocorticoids and responses to such therapy should be evaluated carefully. In the same study, we have shown that 78% of CAD dogs responded adequately to such treatment. In the first stages of the disease, pruritus responds well to the administration of a reduced amount of glucocorticoid (e.g., 0.3-0.5mg/kg prednisolone daily). In chronic cases however, the development of secondary bacterial or yeast infections corresponds to a poorer response to such treatment.

Lastly, we showed that 82% of dogs with CAD spend most of their time indoors. This suggests that prolonged exposure to house dust mites may trigger or worsen clinical signs of CAD.

Clinical signs
Although very frequent, CAD may be difficult to diagnose due to the lack of pathognomonic signs and the protean clinical picture. Erythema and pruritus are usually present and often represent the first clinical signs. However, mild pruritus may remain unrecognized by the owner and the veterinarian may sometimes rely on indirect proof of pruritus such as the presence of excoriations or saliva-coloured hairs.

Most of the clinical signs are actually due to self-trauma and/or secondary infections. In fact, small erythematous papules, which are considered the primary lesion of CAD, are rarely
observed in dogs with the disease. The practitioner will usually observe the consequences of
the inflammation and pruritus, namely excoriations and self-induced alopecia and/or the signs
of the secondary bacterial infection (papules, pustules, crusts, erosions) and/or the signs of
secondary yeast dermatitis (epidermal hyperplasia, hyperpigmentation, lichenification).
Recurrent or chronic skin or ear infections are frequently observed in dogs with CAD. In our
study, bacterial infections were observed in 66% of the patients, while yeast dermatitis and
otitis externa were present in 33% and 50% of all affected dogs, respectively.

However, most of these clinical signs are not specific and the distribution of the lesions is
more helpful. The most commonly affected areas are the pinnae (58%), axillae (62%),
abdomen (66%), front (79%) and hind (75%) feet, lips (42%) and the perineal area (43%).
Unfortunately, these areas are rarely affected simultaneously in the same individual, except in
chronic cases.

Dermatological (pyotraumatic dermatitis, interdigital fistulae) and non-dermatological signs
are sometimes associated with CAD and their presence should reinforce suspicion of the
disease. For example, spring/summer conjunctivitis is found in approximately 20% of dogs
with CAD, while gastrointestinal signs (soft stools, diarrhea, vomiting) are recognized in
26% of dogs with food-induced disease (FIAD).

Clinical signs in dogs with FIAD differ very slightly from those of classical AD. In our study,
statistically significant differences were only uncovered for gastrointestinal signs,
seasonality, corticosteroid-responsive pruritus and pruritus sine materia (less frequent in dogs
with FIAD). Interestingly, dogs with FIAD often have the initial clinical signs early in life
(less than one year) or rather later in life (more than 6 years of age).

**Diagnosis of CAD**

CAD is a diagnosis of exclusion
The diagnosis of CAD is based on the history (age at onset, seasonality, pruritus without skin
lesions at onset, familial or breed predisposition, previous response to glucocorticoids),
development of the disease (seasonality, “wax and wane” character, development of
secondary skin infections) and the pattern of lesions. A diagnosis of CAD should never be
made when diseases such as flea infestation, sarcoptic mange and primary skin infections
(bacterial and fungal) have not been ruled out. Depending on the clinical presentation and the
age of the affected dog, some other differentials include demodicosis, dermatophytosis,
cheyletiellosis and cutaneous lymphoma. The histopathological findings in patients with
allergic skin disease are usually not specific and skin biopsies are not typically adequate to
make the diagnosis. Skin biopsies may be indicated in some instances to rule out diseases
such as cutaneous lymphoma.

Allergy testing (serological evaluation of allergen-specific IgE and intradermal skin testing)
is not regarded as criteria for the diagnosis of CAD. This is because numerous healthy dogs
are sensitized to environmental allergens and are consequently positive (poor specificity).
Dogs with ALD and some dogs with FIAD are deemed negative. These tests should be only
used to identify the offending allergens (i.e., to choose the allergens for allergen-specific
immune therapy [desensitization]). In order to identify dogs with FIAD, an appropriate 8-week elimination food trial should be carried out in all dogs with clinical signs of CAD. It has recently been shown that 90% of the dogs with food allergy improve within 8 weeks during an appropriate trial. It may be necessary to continue the new food in some dogs.

Criteria do not replace thorough diagnostic evaluation

Several sets of criteria have been proposed for the diagnosis of CAD. These criteria are mainly used for clinical trials to increase homogeneity of the recruited dogs. We recently performed a study in which 1,800 pruritic dogs were evaluated by experienced dermatologists throughout the world. One of the goals of the study was to identify criteria for the diagnosis of CAD. The best set of criteria has been associated with a sensitivity and specificity of about 80%, when 5 out of 8 criteria are fulfilled (see table). This means that using these criteria as the sole diagnostic criteria would lead to an inappropriate diagnosis in a fifth of the patients. These data confirm that ruling out resembling diseases should always be a compulsory prerequisite for the diagnosis of CAD.

The place of “allergy tests” in evaluation of dogs with suspected CAD

As mentioned above, allergen-specific IgE tests cannot be regarded as “allergy tests”, because negative results may be found in some atopic dogs and positive results are often found in healthy individuals. These tests are only useful to identify the allergens potentially involved in the allergic reaction in dogs with CAD. In this regard, it is impossible to compare skin tests (intradermal tests) and blood tests (IgE serology), or to determine whether one allergen provider or one lab is better than the others! In one study, outcomes of allergen-specific immunotherapy based on serology or skin testing were similar. These tests are only useful for the determination of sensitivity to environmental allergens but are not reliable for food allergens. For this diagnosis, a study has shown that patch testing is better than skin tests or food allergen-specific IgE serology. As well, some promising results have been obtained using lymphocyte stimulation or basophil degranulation. The former tests are difficult to carry out in daily practice and the latter could only be made in specialized labs. Recently, a western-blot based test has been proposed in several European countries. This test is marketed for the choice of food for dietary trials and not for the diagnosis of food allergy. The accuracy of this test for the latter indication is still under evaluation.

Conclusions

The diagnosis of CAD is one of the most difficult in veterinary dermatology. This is because no pathognomonic signs are known and because of the etiological and phenotypical variability of the disease. For this reason, only a thorough work-up can guaranty a proper diagnosis. It should be kept in mind that no diagnostic test or sets of criteria can effectively replace this thorough work-up!
Criteria | Use | Reliability
--- | --- | ---
**Set 1**
- Age at onset <3 years
- Mostly indoor
- Corticosteroid-responsive pruritus
- Chronic or recurrent yeast infections
- Affected front feet
- Affected ear pinnae
- Non-affected ear margins
- Non-affected dorso-lumbar area

• Use for clinical studies and adapt required criteria based on goal of the study

- Sensitivity 85.4%
- Specificity 79.1%

• If higher specificity is required, 6 criteria should be fulfilled (e.g., drug trials with potential side effects)

- Sensitivity 58.2%
- Specificity 88.5%

• Use to evaluate the probability of the diagnosis of CAD

5 criteria:
- Sensitivity 77.2%
- Specificity 83%

• Do not use alone for CAD diagnosis and rule out resembling diseases

6 criteria:
- Sensitivity 42%
- Specificity 93.7%


Selected References
1. Introduction

Canine atopic dermatitis is a complex and multifactorial clinical syndrome linked to a combination of genetic and environmental factors. Although we have traditionally referred to atopic dermatitis as one disease it is much more likely that is a syndrome in which the characteristic clinical signs may be achieved through multiple and somewhat overlapping pathways. This may be one of the reasons for which we have struggled to identify one specific biomarker and one targeted treatment that is effective in all cases. The traditional approach has been to address the inflammatory process associated with atopic dermatitis and the frequent link with allergic sensitization. Treatments that have been found to be most effective are broad spectrum therapies that decrease a multitude of cytokines and inflammatory mediators but that may also cause adverse effects such as increased risk of infections. It is important to note, however, that atopic dermatitis clinical signs do not need to always be linked to an allergic process. As we have increased our understanding on the pathogenesis of this complex disease in the last decade, we have become very aware of the importance of skin barrier dysfunction, whether primary or secondary to inflammation. This skin impairment can play an important role in facilitating allergen penetration and increasing the risk for allergic sensitization thus some of the more recent therapies have been aimed at restoration of skin barrier impairment. Many atopic patients have several trigger factors ranging from multiple allergies and secondary bacterial and yeasts infections, all contributing to the inflammation and the pruritus. Thus, as part of the treatment, it is important to control the trigger factors and bring the patient below a threshold of clinically appreciable pruritus.

As the disease has a life-long, chronic relapsing course with periods of remission and exacerbation, the treatment should be aimed at both controlling the acute flares as well as having a long term plan on how to decrease the likelihood of new flares. Depending on the mechanism and the speed of action of the treatment, different approaches may be used for the acute versus the chronic management. It is also important to highlight the fact that such a complex disease is best managed with a multimodal approach which needs to be tailored to each individual case.

2. Management of acute flares

Acute flares are best handled by 1) identifying the most immediate trigger factors (e.g. fleas, specific foods) and 2) using a treatment that can provide immediate relief decreasing inflammation and pruritus. The persistence of inflammation and the skin damage caused by self-trauma can have rapidly negative effects not only on the comfort level of the animals but also to the development of the secondary infections, which can further complicate and increase the severity of the clinical signs. While glucocorticoids have been used for a long time and are well known treatment with its pros and cons, the use of a JAK inhibitor like oclacitinib, is still relatively new. Oclacitinib has been demonstrated to be effective and rapid
in providing relief to affected animals and it is considered a suitable alternative to the use of glucocorticoids to provide fast control of clinical signs. The recommended regimen is 0.4-0.6mg/kg orally twice daily for the first 2 weeks followed by once daily for subsequent therapies. Although oclacitinib may not work in all atopic patients, it is overall a very effective therapy and well tolerated treatment. Many patients may show worsening of clinical signs when switched from the twice daily to the once daily treatment which typically levels out overtime. Besides the lack of adverse effects like polyuria and polydipsia oclacitinib has another advantage over glucocorticoids as it does not appear to have a negative impact on intradermal skin test thus it could be used short term to make the patient comfortable while working up the triggers like environmental allergens. Unfortunately, the benefits of relief provided by oclacitinib are typically short lived once the medication is discontinued and clinical signs rapidly return, sometimes even at a higher level than before the initiation of therapy (rebound). It is important to note that oclacitinib is not approved for use in dogs younger than 12 months of age thus cannot be used in really young atopic patients.

For patients that respond to oclacitinib but are incompletely controlled at the once daily regimen it may be useful to consider the subcutaneous injection of the caninized anti-cIL-31 monoclonal antibody from Zoetis that has received conditional approval for use in dogs. This is the first biologic available in veterinary dermatology and it is aimed at blocking IL-31, which is a mediator of pruritus in dogs. Whether IL-31 is a main cytokine in canine atopic dermatitis is still unclear. One study had failed to detect IL-31 in the skin of atopic dogs altogether and another study detected IL-31 in the serum of only 57% of atopic dogs. Thus, although IL-31 injection can induce pruritus in dogs, it is not clear that this is a critical cytokine in atopic dermatitis. Based on preliminary results provided by Zoetis, IL-31 appears to provide relief in 80% of pruritic atopic patients and is supposed to last for a full month. Initial clinical experience at UF has showed some positive response but not to the degree and duration described. At this point, this biologic would not be considered as the first line of treatment for atopic dogs and other strategies with more evidence of efficacy should be considered.

As part of the acute flare management it is important to use topical therapy as adjunctive strategy to decrease pruritus and sooth the skin. Topical glucocorticoids can also be used to provide fast relief, particularly in patients with localized disease. If infections are present, they need to be addressed to decrease pruritus and allow maximum benefit of anti-inflammatory treatments.

3. Medium to long term management
For the medium term control of the disease many clinicians chose to use cyclosporine. The benefit of cyclosporine therapy is not evident for the first 3-4 weeks thus this type of approach requires the use of another faster acting therapy while waiting for the benefit of cyclosporine. As other immunomodulating therapies, cyclosporine may increase the risk for infections when used for prolonged periods of time. Despite this, cyclosporine is considered overall a safe treatment for medium to long term use. The most common adverse effect when prescribed at 5mg/kg once daily is gastrointestinal ranging from vomiting to diarrhea and
decreased appetite. In some dogs a papillomatous dermatitis may develop which is typically responsive to a decrease of dose and antibiotic therapy.

Oclacitinib can also be used for long term therapy as well, and it appears to be safe. However, for patients requiring medication for many months to years it is always prudent to find alternative therapies when treatment is needed for extended periods of time. The long term strategy is typically composed of anti-inflammatory treatments (e.g. cyclosporine, oclacitinib or glucocorticoids) in combination with immunotherapy to modulate the hypersensitivity reaction and minimize future flare ups.

Allergen specific immunotherapy is still considered the best long term approach for young animals with symptoms present for many months to years. Although allergen specific immunotherapy is typically presented as “expensive”, when compared to the cumulative cost of other forms of management (e.g. cyclosporine in a large breed dog) it is actually cost effective as it can decrease the frequency of infections, therefore, the use of antibiotics and the risk of resistance as well as the need for other medications. In human medicine, allergen specific immunotherapy has been demonstrated to alter the course of the disease and decrease the number of sensitizations in the long run. Whether this is applicable to dogs is currently unknown. Despite the fact that allergen specific immunotherapy has been used for many years, there are few published studies particularly controlled ones. Insufficient information is currently available to indicate which protocol is the best one although it appears that higher dose and allergen specific seems to provide better results rather than lower dose and pre-mixed. While the traditional route of administration for allergen specific immunotherapy has been the subcutaneous route, more recent studies have also showed safety and efficacy of sublingual immunotherapy. This route should be considered in patients that have had adverse effects with the injections. The improvement with sublingual immunotherapy may be noticeable in the first 6 months and, therefore, may be considered faster than the traditional subcutaneous injections. Most patients require adjunctive therapy in the first few months of therapy; therefore, drugs like glucocorticoids, cyclosporine, or oclacitinib may be used while building up the allergen specific immunotherapy. An indirect measurement of effect is noticed by the decreased need to medications to maintain control of signs.

An approach accepted in human medicine to reduce flare ups is the proactive treatment of areas where the patient typically develops lesions, even when the skin appears to be clinically normal. This approach has been tested in a small randomized controlled study in dogs with atopic dermatitis with encouraging results. In this trial, hydrocortisone aceponate spray was applied to areas prone to lesions two days/week and that led to 4 times longer relapse time. Monitoring of cutaneous atrophy should be considered in the long term.

Finally, the use of essential fatty acids, either orally or topically should be integrated in the long term management of atopic patients. This type of supplementation requires time to produce a beneficial effect but it has been proven to increase and restore some of the lipid abnormalities in the epidermis. Topical application of sphingolipid emulsions can also lead to improvement and should be considered as part of adjunctive therapy rather than monotherapy. Similar consideration is for the use of antihistamines which are best used
before the beginning of the allergy season and more with the goal of adjunctive therapy to minimize the need for other medications rather than a rescue drug once an acute flare up has developed.

In conclusion, the management of atopic dermatitis is multimodal and should be tailored to the individual patient considering the age, the duration of symptoms and the expectations of the owners. Although atopic dermatitis cannot be cured, much progress has been done in recent years and more treatment options are available to improve the quality of life of affected patients.

Selected References
DIAGNOSTIC APPROACH TO OTITIS EXTERNA

Tim Nuttall

Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Roslin, United Kingdom

1. Introduction
Otitis externa is very common. The initial diagnosis is relatively straightforward, but it is important to undertake further examination and tests to identify the likely organisms and extent of inflammation to select the most appropriate treatment. It is also important to determine the primary, predisposing, perpetuating and secondary factors in cases of chronic and recurrent otitis. Diagnostic steps include:

- Looking at the signalment and taking a good history
- Thorough clinical examination
- Otoscopic examination and (if possible) video otoscopy
- Cytology
- Culture and antimicrobial susceptibility testing
- Imaging – radiographs, CT and/or MRI
- Biopsy and histopathology

2. Signalment and history

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3. Clinical examination
A. Erythroceruminous otitis
This is most common and presents with erythema and a ceruminous discharge; erosions and ulcers are rare. The inflammation, discharge and chronic pathological changes vary from mild to very severe. The ears are usually more pruritic than painful. Staphylococci and Malassezia are the most common organisms. The exudates are usually yellow-brown with bacteria and chocolate brown with Malassezia, although there is overlap and mixed overgrowths are seen.

B. Suppurative otitis
These show pain, inflammation, ulceration, hemorrhage and a purulent (usually yellow to green and foul smelling). Pseudomonas is most common. Proteus, other Gram-negative bacteria and occasionally staphylococci or streptococci can also be found. Malassezia is rare.

C. Clinical features of common primary causes of otitis
i) Otodectes are associated with large amounts of dry, dark brown, waxy debris. Mites may be seen moving in the canal or on microscopic examination of cerumen. They may be difficult to find in some individuals that may have a hypersensitivity and low mite numbers.

ii) Atopic dermatitis and adverse food reactions are the most common cause of recurrent or chronic otitis in dogs; the muzzle, feet and ventral body are also affected in most cases, although recurrent otitis may be the only clinical sign. There is a diffuse erythema of the ventral pinna and vertical ear canal. Up to 80% of dogs with hypersensitivity will suffer recurrent otitis. Eosinophilic granuloma syndrome lesions may be seen in the ear canals of cats, with or without lesions elsewhere.

iii) Contact reactions to topical ear medications result in ongoing inflammation and a white to purulent discharge with mature non-degenerate neutrophils but few organisms. There is often a history of an initial response followed by relapse and pain on administration.

iv) Immunosuppressive diseases, including hypothyroidism and hyperadrenocorticism can also trigger otitis and other skin infections, which are often an early sign of the underlying problem. Sex hormone alopecias are associated with seborrhoeic otitis externa.

v) Keratinisation disorders will result in scaling and seborrhoea of the pinnae and ear canals.

vi) Immune-mediated diseases can cause ulceration and inflammation of the pinnae and, less commonly, the ear canals, but other clinical signs are usually more prominent. Pemphigus foliaceus is the most common - pustules on the concave aspect of the pinna are a good indication. An immune-mediated ulcerating disease of unknown aetiology affecting the ear canals has been recognised. Punched out ulcers and notches can be associated with vasculitis. Juvenile cellulitis causes severe oedematous otitis in young puppies.

vii) Ceruminous gland tumours can obstruct the ear canal and cause otitis before the tumour is visible. Most are benign, but adenocarcinomas are locally invasive and can metastasise. Rare tumours include melanomas, haemangiosarcoma and basal
cell tumours. External tumours can compress, infiltrate and obstruct the middle ears and ear canals.

viii) Foreign bodies (e.g. grass seeds) are usually easily seen and removed in the clinic, but might need flushing out under sedation.

ix) Feline naso-pharyngeal polyps may originate from the naso-pharyngeal, auditory tube or middle ear mucosa. Their aetiology is unclear; immune-mediated, congenital and post-viral causes are possible. Most have otitis media with or without otitis externa.

x) Ceruminous/sebaceous hyperplasia is a frustrating condition seen in Spaniels (which have increased numbers of glands in their ear canals), and occasionally other breeds and cats. These are difficult to manage and often develop chronic pathologic changes.

D. Chronic pathological changes
The ear canals should be carefully palpated and inspected to determine the nature and degree of chronic pathology changes, which include:

i) Hyperplasia of the ceruminous glands, increased cerumen and increased humidity

ii) Thickening of the dermis and epidermis leading to ear canal stenosis

iii) Chronic fibrosis and ossification of the ear canals

iv) The tympanic membrane may rupture and reform with diverticula and cholesteatoma

4. Otoscopic examination
Most dogs accept this with no sedation. Insert the cone into the vertical canal and pull the pinna out and ventrally as you move the cone into the horizontal canal – this straightens the ear canals and flattens the ridge at the base of the vertical canal. Clean the ears or use anti-inflammatory treatment to open stenosed ears if necessary. Use sedation or anaesthesia in fractious animals or painful cases; struggling makes it difficult to see anything and you may damage the ear. In addition, the otoscope cone can absorb much of the light leading to poor illumination of the ear canal. Passing instruments down the cone will obscure the view.

Careful inspection can identify foreign bodies, Otodectes, Otobius ticks, inflammation, ulceration, stenosis, the condition of the tympanic membrane, exudation and chronic changes. Healthy ear canals have a thin, smooth, pale pink lining. There should be a small amount of waxy pale yellow-brown cerumen. The tympanic membrane should be thin, translucent, sloping ventrally and slightly concave.

Acute inflammation results in moist erythematous swelling. Chronic changes have a firm indurated surface. Ceruminous hyperplasia results in a ‘cobblestone’ appearance. In some dogs this may develop into single or multiple polyp-like growths. The discharge is usually more aqueous or seborrhoeic. The tympanic membrane may be torn, inflamed, discoloured, thickened and/or convex, although it is difficult to visualise with a lot of debris or stenosis. The integrity of the tympanic membrane can be assessed by gently probing with a soft tube. Debris can mimic the membrane, but tubes pass into the middle ear without resistance. If the
membrane is not visible, use anatomical cues (e.g. the hairs at the ventral insertion of the tympanic membrane and the bony external auditory process) to check that you are at the right level.

5. Video otoscopy
Video otoscopes are less convenient, but have many advantages. The magnification, illumination and image quality allow assessment of fine detail. The working channels greatly facilitate ear flushing, foreign body removal, traction of polyps, minor surgery, laser procedures and myringotomy. Image and video capture improves client communication and helps with follow up of cases.

6. Nature of the discharge

<table>
<thead>
<tr>
<th>Colour</th>
<th>Dark brown</th>
<th>Pale brown to grey</th>
<th>Pale brown to yellow</th>
<th>Yellow to green</th>
<th>Dark green to black</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consistency</td>
<td>Waxy and adherent</td>
<td>Waxy to seborrhoeic</td>
<td>Seborrhoeic to purulent</td>
<td>Purulent</td>
<td>Thick and slimy</td>
</tr>
<tr>
<td>Association</td>
<td>Ceruminous otitis</td>
<td>Malassezia</td>
<td>Staphylococci</td>
<td>Pseudomonas</td>
<td>Biofilm</td>
</tr>
</tbody>
</table>

Just looking at the dried material at the opening of vertical ear canal can be misleading and the discharge should always be assessed by otoscopy or taking a sample from the ear canals. These findings are indicative only and cytology should be performed wherever possible.

7. Cytology
Cytology of the exudate should be performed in all cases. Where appropriate, samples should be taken from the external ear canals and middle ear as the results may differ. Samples may also taken from specific lesions, e.g. polyps, nodules etc. Debris can be collected using cotton buds, catheters, spatulas, curettes or loops (rubber or plastic are preferred in conscious animals to avoid trauma). It can be mixed into liquid paraffin or potassium hydroxide to look for mites. Adhesive tape can be used to collect material from the pinna.

For cytology, gently roll or blot the material onto a slide to form a thin layer (don’t smear samples as this ruptures the cells), and then air dry and stain (heat fixing may be necessary with very oily samples). Diff-Quik® or Rapi-Diff® type stains are quick and easy, and allow identification of most bacteria, yeasts, leukocytes, mononuclear cells, squames, acanthocytes and neoplastic cells. Adhesive tape strips should not placed in the fixative (pot 1), as this may dissolve the adhesive layer or turn the tape opaque. The one-stain method using only the basophilic stain (pot 3) can be very effective for staining microorganisms in thick and greasy preparations. A single drop of the stain is placed on the smear before adding a coverslip, or a single drop is placed on a slide before pressing the tape on.

Non-nucleated keratinocytes are seen in all ears, but there are increased numbers of nucleated and non-nucleated cells in chronically inflamed ears with hyperkeratosis. Large coci in pairs or cluster suggest *Staphylococcus*, whereas *Streptococcus* and *Enterococcus* are a little
smaller and tend to form chains. Long narrow rod bacteria are typical of *Pseudomonas*, while *Proteus* and other Gram-negative species tend to be shorter or bipolar. All bacteria that stain with Diff-Quik® type stains will stain dark blue, and Gram-stains are necessary to distinguish them. Peanut-shaped yeasts are characteristic of *Malassezia*.

Most cocci and *Malassezia* infections are associated with overgrowth in the absence of neutrophils. Large numbers of degenerate neutrophils with intracellular bacteria are usually seen with *Pseudomonas*, and occasionally more severe staphylococcal infections. Neutrophils are also seen in contact reactions, and both red blood cells and neutrophils are common with ulceration of the ear canal. Mucoid slime is evident with biofilm forming organisms.

It is possible to obtain material for indirect impression smears or fine needle aspirates from lesions in the ear canals using fine swabs, curettes or long needles passed through an otoscope. This is best done using the working channel of a video otoscope.

8. Bacterial culture and antibiotic sensitivity
This is not always required. Microorganisms are easily identified on cytology, and this can tell you which organisms are most important in mixed infections. *Malassezia* and staphylococci have a predictable sensitivity, but gram-negative rods (especially *Pseudomonas*) are frequently resistant to many antibiotics. Any ear not responding to treatment as expected should also be cultured, taking material from both the ear canals and middle ear if necessary. Antibiotic sensitivity data is less useful with topical therapy as the concentrations achieved in the ear canal will be be ~1-4,000x that predicted by *in vitro* sensitivity tests. This can overcome resistance, and the response to treatment is best assessed by clinical signs and cytology.

9. Biopsy and histopathology
It is usually impossible to get adequate biopsies of the ear canal epidermis with endoscopic biopsy forceps designed for mucosal surfaces. Material can be collected using shaves, traction and 4mm biopsy punches. This will help identify neoplasia, immune-mediated diseases, and the nature and extent of chronic pathological changes.

10. Diagnostic imaging
Diagnostic imaging is primarily used to investigate otitis media, but it can also be helpful in some cases of otitis externa. It’s possible to perform this using sedation but anesthesia will facilitate positioning in most cases, and is required for MRI scans.

In dorso-ventral or ventro-dorsal the ear canals should be visible and air-filled. Soft-tissue filling indicates occlusion with debris or stenosis. Mineralisation of the ear canals may be seen in severe cases. Diluted soluble contrast material (e.g. 50% Hypaque® diluted 1:10 in saline) can be used to delineate the canals the test the tympanic membrane – if it is ruptured contrast leaks into the middle ear. However, the fluid may not leak through small tears. Stenosis, in contrast, can block the flow falsely suggesting the membrane is intact. This be alleviated somewhat by introducing the contrast agent through a catheter directing into the horizontal ear canal to fill the rest of the ear canal by retrograde flow. Massaging the ear
canals will also help distribute the material. Cotton wool plugs will prevent the contrast material leaking on the skin or surrounding surfaces.

CT is very sensitive and specific for stenosis and occlusion of the ear canals, bulging or rupture of the tympanic membrane. CT is more expensive but is quicker and easier to perform and interpret. The ear canals should be thin-walled, open and of uniform diameter or gently tapered towards the tympanic membrane. The density of the material in the ear canals can be assessed to help differentiate debris from stenosis, and contrast enhancement can be used to identify inflamed soft-tissues. Any amount of mineralisation of the ear canals is easily seen. MRI is less good for imaging the ear canals but can be useful for imaging soft-tissue structures such as neoplasia, infection and nerves in or around the ears.

11. Tympanometry
This is used to assess the integrity of the tympanic membrane. Air is introduced into the ear canal via a closed otoscope. The tympanic membrane should flex back and forth; reduced movement or a bulging membrane suggests fluid or debris in the middle ear. This is difficult technique to perform and assess, and it is not commonly done. Auditory tympanometry can be used to generate acoustic waveforms to objectively assess the compliance of the tympanic membrane and pressure in the middle ear, although it can be difficult to perform in animals.

12. Brainstem auditory evoked responses (BAER)
BAER tests are the only way to objectively assess hearing and the only way to identify unilateral deafness. It is quick and well tolerated, although sedation may be required in some animals. Anaesthesia is not usually necessary. Headphones or earplugs are used to deliver brief clicks at different rates, frequency and intensity. The evoked responses are detected by three subcutaneous electrodes and converted into a BAER trace with 4-6 waveforms. The structure of the waveform can be used to identify abnormalities in the middle ear, cochlea or central auditory pathways. Flat traces indicate impaired hearing and the intensity of sound (decibels) that can be heard. Bone stimulation can be used to differentiate conductive hearing loss from neural deafness, although this usually requires sedation and is harder to perform and interpret.

Selected References
OTITIS EXTERNA: INTERACTIVE CLINICAL CASES

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There are no written proceedings for this session — please use this space for your own notes.
RATIONAL ANTIMICROBIAL USE: CRITICAL STEPS IN DECISION MAKING

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Antibiotics are essential to cure bacterial infections but their use promotes selection of resistant bacteria, thereby contributing to reduced antibiotic efficacy over time. Even though resistance is a natural phenomenon, resistant bacteria are selected (not created) by antibiotic use. It is impossible to eradicate antibiotic resistance unless we stop using antibiotics. However, we can control and to some extent prevent clinical challenges related to antibiotic resistance by using them in a rational way. Rational antimicrobial use is a term that comprises any actions that contribute to maximize the clinical efficacy of antibiotics and/or to prevent resistance in the strain causing infection, as well as, in the patient’s commensal microbiota. Antibiotic choice is a cornerstone of rational antimicrobial use as both therapeutic efficacy and prevention of resistance are strongly influenced by the type of drug prescribed/used. Critical decisions on antibiotic choice are taken at two different steps in the diagnostic process: the first (empirical) immediately after clinical examination of the patient and the second two to three days later, once laboratory results of culture and sensitivity testing have become available. The critical decisions to be taken in the first visit can be summarized with four questions:

Question 1. Is a systemic antibiotic needed?
Systemic antibiotics should only be used if bacterial infection is suspected on the basis of well-grounded clinical data. Before using or prescribing systemic antibiotics the veterinarian should consider the possibility that infection is self-limiting (i.e., infections that resolve spontaneously with or without specific treatment) or caused by viruses or parasites. This is the case for most upper respiratory and enteric infections. In veterinary dermatology, systemic antibiotics can be replaced by antiseptics, which may have comparable therapeutic efficacy and do not select for resistance in the commensal microbiota outside the application site, especially in the gut where most bacteria and opportunistic pathogens reside.

Question 2. Is empiric antibiotic therapy needed?
Empiric therapy is recommended if infection is life-threatening or causing pain or discomfort in the patient, and if delay in treatment could adversely affect the clinical outcome. Culture is almost never contraindicated except if collection of a suitable clinical specimen requires invasive procedures that may complicate infection or patient stability, or if interpretation of the culture result is hampered by contamination with commensal bacteria. In veterinary dermatology, culture and antibiotic susceptibility testing is of little use for those infections that are managed topically such as otitis externa, wound infections and some forms of superficial pyoderma. For skin infections that require long-term systemic antibiotic therapy, culture and susceptibility testing is recommended, especially in countries with high prevalence of methicillin-resistant *Staphylococcus pseudintermedius* (MRSP). When samples
are sent to the laboratory for culture and susceptibility testing, local treatment with antiseptic products can be initiated while waiting for the laboratory results in order to choose the most appropriate antibiotic based on the susceptibility profile of the infecting strain, thereby avoiding empiric therapy.

Question 3. If empiric therapy is needed, which antibiotic should be used/prescribed? A qualified choice requires basic knowledge of the pharmacology of antibiotics, of the causative agents of bacterial infections in companion animals and of the local patterns of antibiotic resistance. In particular, the drug should be able to penetrate and be active at the infection site, active on the most likely bacterial species suspected to be responsible for infection, be non-toxic to the patient, easy to administrate, and as narrow spectrum as possible. With regard to the last point, empiric therapy with broad-spectrum drugs such as 3rd generation cephalosporins or fluoroquinolones should be avoided unless the infection is life-threatening or is comprised among those infections for which one of these drugs is recommended as first choice by national and international guidelines for antimicrobial use (e.g., fluoroquinolones are recommended as first choice in the management of acute or chronic prostatitis due to their ability to pass the blood-prostate barrier). In other situations, the choice should fall on drugs that have narrower spectrum, since broad spectrum cephalosporins and fluoroquinolones have a considerable impact on the commensal flora and are likely to promote selection of multidrug-resistant bacteria. For certain types of infections (e.g., otitis, skin infections and UTIs), antibiotic choice should be guided by cytology, which reveals whether the pathogens involved are Gram-positive cocci or Gram-negative rods. Local patterns of resistance may be gathered from national reports, scientific articles or, even better, from retrospective analysis of the susceptibility data at the clinic level.

Question 4. Regardless if empiric therapy is initiated or not, should a clinical specimen be submitted to the microbiology laboratory? Even if empiric therapy is initiated, culture and antibiotic susceptibility testing are recommended if (i) there is suspicion of a complicated infection (i.e., an infection associated with structural or functional abnormalities or presence of underlying disease, which increases the risks of failing therapy, (ii) the patient has not responded to therapy or has a history of relapse or re-infection or (iii) there is any reason to suspect infection with multidrug-resistant bacteria such as MRSP or MRSA on the basis of anamnesis and clinical records. Culture and susceptibility testing are necessary if the patient is immunocompromised or the infection is life-threatening.

A clear distinction should be made between empirical choice and choice based on susceptibility testing results. This important distinction is largely overlooked in most veterinary guidelines for antimicrobial use, which usually only provide recommendations on antibiotic choice for empiric therapy. Once laboratory results of culture and sensitivity testing have become available, antibiotic choice may be complicated by several factors that can be summarized by another set of questions:
Question 5. Which antibiotic to choose based on susceptibility data?
When choosing an antibiotic based on susceptibility data, the choice should fall on the drug that has the least possible impact on selection of multidrug-resistant bacteria, provided that the drug is clinically effective and non-toxic. Off-label use of products registered for human use should only be considered if the test strain is resistant to all antibiotics licensed for veterinary use. Interpretation of antibiotic susceptibility reports is not as simple as it may appear on the surface. Some of the antibiotics used in clinical practice are not included in the antibiotic panel tested by the microbiology laboratory because they lack approved clinical breakpoints (i.e., the threshold values used to categorize strains as resistant, intermediate or susceptible). An example would be the antibiotic cefovecin. In the absence of clinical breakpoints, surrogate drugs may be used to predict the efficacy of other drugs belonging to the same antibiotic class and displaying similar pharmacodynamics and pharmacokinetic properties. Thus, it is important to know which antibiotics are used as surrogate drugs. On the other hand, some drugs in the antibiotic panel are not used in clinical practice. This is the case of the antibiotics that are used by the laboratory for detection of MRSA and MRSP (i.e., oxacillin and cefoxitin). Finally, interpretation may be complicated when the laboratory report includes susceptibility profiles of multiple strains. Some infections, mainly wound infections and otitis externa, often result in culture of multiple bacteria. In these situations, targeting the primary pathogen is the most reasonable approach since targeting all the strains cultured may be difficult and lead to unnecessary use of broad-spectrum drugs. The clinical relevance of each organism reported by the laboratory should be considered based on its pathogenicity. For example, Corynebacterium auriscanis is unlikely to be a primary pathogen in otitis externa as it is never isolated alone. Anecdotal evidence suggests that otitis externa associated with this organism resolves if the primary pathogen is targeted by antibiotic therapy. Coagulase-negative staphylococci (skin contamination), Bacillus spp. (soil contamination) and enteric bacteria (faecal contamination) are among the most common « contaminants » of veterinary dermatology clinical specimens.

Question 6. Should therapy be changed if the strain is reported as resistant to the antibiotic that was prescribed empirically?
In theory, the initial therapy should be interrupted and a new drug should be chosen among those to which the strain is susceptible. However, this is not necessarily a wise decision since various studies have shown that the therapeutic outcome is not always predicted by in vitro susceptibility testing and infection can be eradicated even if the causative agent is reported as resistant. Thus, patient’s conditions and treatment outcome should always be checked before changing antibiotic therapy based on susceptibility reports.

In addition to antibiotic choice, other key aspects of rational antimicrobial use include dose, administration interval and treatment duration. Here a different set of questions needs to be answered:

Question 7. What is the most appropriate dose?
As a matter of principle the dose should follow the label instructions provided by the drug manufacturer. When the label instructions indicate that the drug can be administered at different doses, the highest dose is recommended for concentration-dependent drugs (e.g.,
fluoroquinolones) in order to enhance therapeutic efficacy as well as to prevent selection of resistant mutants.

Question 8. What is the most appropriate administration interval?
The interval at which a drug is administered is particularly important for time-dependent antibiotics such as all β-lactams since therapeutic efficacy is affected if these drugs are not prescribed according to the recommended interval (e.g., q12 or q8). The administration interval also influences prevention of resistance to concentration-dependent drugs since delayed administration may lower the drug concentration below the Mutant Prevention Concentration (MPC), therefore increasing the risk that resistant mutants are selected during therapy.

Question 9. What is the most appropriate treatment duration?
This question is difficult to answer due to knowledge gaps. For some infections the recommended courses of antibiotic therapy in veterinary medicine are significantly longer than for human medicine and this difference is not justified by scientific evidence. The latest trend in human medicine is that unnecessary treatment should be avoided after clinical resolution of symptoms.

Acknowledgements
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MANAGING RECURRENT PYODERMA

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To every veterinarian’s frustration, staphylococcal skin infections can be stubbornly recurrent in some dogs. The client (and the veterinarian) must understand that staphylococcal bacteria are normal flora; infection cannot occur unless something has gone wrong with the skin or its defense systems. Thus, particularly in recurrent infections, the first step is to attempt to define the underlying cause with appropriate diagnostic investigation. In younger dogs with recurrent infections, common causes of recurrence include external parasites and allergic disease. Older animals can also develop recurrent infections from hypothyroidism or any other underlying systemic disease. Despite thorough testing, some patients with recurrent infections defy diagnosis—their infections respond completely to antibiotic treatment, yet continue to recur soon after such treatment is discontinued. For such patients with “idiopathic recurrent pyoderma,” there are several measures that may help to prevent or limit recurrence.

In some cases of recurrent pyoderma, there are complicating factors. We must consider several forms of pyoderma in which additional factors contribute to the pathogenesis and make treatment difficult. Examples include German shepherd dog pyoderma/cellulitis—a special form of deep pyoderma in which there is evidence of a genetically determined cellular immunodeficiency—and interdigital pyoderma, in which, in addition to staphylococcal infection, the deep infection that occurs between the toes is, in part, a foreign-body reaction to hair shafts, perhaps entrapped in scar tissue. Recent evidence suggests that at least some cases of interdigital pyoderma truly begin as cystic structures that become secondarily infected.

Emergence of Methicillin-Resistant Staphylococci: Further Complications

There has been a recent increase in reports of multidrug-resistant staphylococcal strains in canine pyoderma. In some areas of the United States, more than 50% of skin cultures performed at dermatology specialty practices are methicillin-resistant staphylococci (MRS). These strains include resistant *Staphylococcus pseudintermedius* strains (canine infections, referred to as “MRSP”) or resistant *Staphylococcus aureus* strains (human infections, referred to as “MRSA” and, fortunately, much less common). Veterinarians should endeavor to use correct terminology when discussing these infections with clients; incorrectly referring to a canine MRSP infection as “MRSA” may be alarming to the client. If laboratory testing indicates the presence of MRS, the isolate will be *clinically resistant to all penicillins and cephalosporins*.

What is the significance of these organisms? First, if you treat a dog with staphylococcal pyoderma with a beta-lactam antibiotic (cephalosporin or penicillin) and there is limited or no response, *culture and susceptibility testing is now mandatory*. Fortunately, most veterinary strains of MRS are still susceptible to routine antibiotics such as trimethoprim-sulfamethoxazole, clindamycin, or a fluoroquinolone such as enrofloxacin or marbofloxacin. However, it is important to note that it is impossible to predict with any certainty which
antibiotics are indicated without performing a susceptibility test. Empirical “antibiotic hopping” is hazardous, as with each cycle of treatment, multiple drug resistance becomes more likely.

Second, if you do identify an MRS organism, especially if it appears to be very resistant, you should order a staph speciation test, though many laboratories will do this routinely. If you have a patient with MRSP (i.e., the canine strain) in your hospital, you need not have the dog under full isolation procedures, but you should isolate the patient to the extent you can and eliminate traffic from this patient to other dogs in the clinic, especially the surgery and critical care areas. If the organism turns out to be a methicillin-resistant, human-origin S. aureus (MRSA), the owner should be notified of this fact so they can discuss the situation with their own health care provider, and gloves should be worn when examining the patient. This patient is a potential human health hazard and should be considered so until all lesions have completely resolved. The concern here is that without proper precautions, the MRSA could colonize the owner, you, your staff, or others. It is important to understand that merely becoming colonized with MRSA is not inherently dangerous. After all, 3% to 5% of people are already colonized at any given moment, and colonization is dynamic and transient. Where the situation becomes potentially dangerous is if the colonized person becomes injured or immunosuppressed.

Third, the emergence of MRS in the veterinary world suggests that we must redouble our efforts to use antibiotics wisely and judiciously, and reconsider all efforts to use alternative, nonantibiotic treatments, if possible, in the face of recurrent infections.

Strategies for Minimizing Antibiotic Use and Preventing Recurrence

Antimicrobial topicals are the first line of defense with recurrent skin infections. Shampoos or other topical products containing 2% to 4% chlorhexidine appear especially helpful for preventing new lesion development when used once to twice weekly. Any patient with a history of recurrent pyoderma, even if it is bathed infrequently, should have a chlorhexidine-based routine cleansing shampoo. Other ingredients, such as benzoyl peroxide, are also effective but tend to be drying and irritating with prolonged use.

Products that are formulated to remain on the skin may have a longer duration of action on the skin than a shampoo and, in many cases, are easier for the owner to apply frequently. For localized areas, treatment with a lotion or wipe may suffice. For broader regions of the skin, spray-on products, mousse formulations, or “leave-on” conditioner products are recommended. To help prevent relapse of recurrent pyoderma, begin with every-other-day application. If effective, the applications may be tapered down to every 3 to 7 days in many patients. The overall principle here is to limit, to the extent possible, prolonged or repeated courses of antibiotic treatments to minimize the potential for development of antibiotic resistance.

Increasingly, dermatologists understand that it is frequently possible to eliminate active superficial staphylococcal infections from the skin by using topical products as the primary treatment without antibiotics. This can be done as a “safety precaution” to avoid yet another
course of systemic antibiotics; alternatively, it can be used for very resistant MRS strains for which antibiotic choices are limited or nonexistent. For primary treatment of an existing superficial pyoderma, daily treatment is necessary until the infection is cleared, which typically takes 4 weeks or more. Whether used daily as primary treatment or every few days as preventive maintenance, the following ingredients are useful in topical products:

- Mupirocin 2% ointment—applied daily to areas of local infection as a primary treatment; not for prevention.
- Chlorhexidine—spray or mousse formulation, for treatment or prevention.
- Nisin Wipes—nisin is an antimicrobial peptide that is commonly used in some areas of the world as a disinfectant teat wipe for dairy cattle and is even used as a food preservative. Preliminary studies in dogs demonstrated that use of these wipes twice daily could limit bacterial colonization and slightly accelerate healing of existing pyoderma in addition to having potential as preventive therapy.
- Use of oxidizing disinfectants such as very dilute sodium hypochlorite solutions (“bleach baths”) has become very popular in human atopic dermatitis, as this treatment greatly helps to limit secondary bacterial colonization of skin. Veterinary products with similar actions (basically solutions with stabilized hypochlorite ions, usually in spray or shampoo formulation) are gaining popularity, although critical studies are lacking.
- Peroxide is an excellent oxidizing disinfectant. The most recently popular products contain “accelerated hydrogen peroxide,” which is simply hydrogen peroxide with added stabilizers and surfactants to enhance its efficacy. Again, we await critical “on-patient” studies of efficacy for these products.

Immunomodulatory therapy can be remarkably effective for some patients with idiopathic recurrent superficial pyoderma. Its use for recurrent deep pyoderma, or for recurrent pyoderma associated with allergic disease, is less well studied. In particular, staphylococcal bacterin products are very useful. These “staph vaccines” are either available commercially (SPL®, Delmont) or are prepared by a local laboratory as autogenous bacterins. They generally must be used long-term to prevent recurrence; however, their use avoids the necessity of prolonged antibiotic treatment in some pets. SPL has a variety of immunomodulatory actions; unfortunately, these have mostly been studied in mouse models or in vitro and rarely in dogs. Recent gene-expression microarray studies in dogs suggest that SPL may exert its effect via up-regulation of interferon-gamma production. SPL is administered at 0.5 cc subcutaneously, twice weekly, for a trial period of 10 weeks. During the first 6 weeks of injections, antibiotics are administered concurrently. After 6 weeks, the antibiotics are stopped and the injections continued. Success is manifested as failure to relapse, much milder relapse, or infrequent relapse compared with before use of the SPL. If SPL is effective, it can usually be reduced to once-weekly injection, and sometimes once every 2 weeks.

Continuous antibiotic treatment via “pulse therapy” has always been a last-resort treatment for recurrent pyoderma, but with the current resistance situation, it should be avoided at all
costs. The emergence of MRS has virtually guaranteed that such treatment will eventually result in colonization by a resistant strain, a phenomenon that is growing worldwide.

Selected References
MALASEZIA DERMATITIS - HOW DO I MANAGE THIS?

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1. Introduction
Malassezia dermatitis is common in dogs, and less common in cats.1-2 Malassezia spp. are commensal yeasts that usually colonize the superficial epidermis of the skin and ears. The non-lipid dependent M. pachydermatis and the lipid dependent M. sympodialis, M. globosa, M. nana, M. slooffiae, M. restricta and M. furfur are usually found on the skin or mucosae of dogs and cats.1-3 Because Malassezia sp. do not invade subcorneally, it is hypothesized that the dermatitis results from inflammatory and/or hypersensitivity reactions to yeast enzymatic products and antigens.4 Malassezia-specific IgG and IgE seem to be higher in atopic then normal dogs pointing toward a possible involvement in the pathogenesis of atopic dermatitis.5,6 This was also shown in human beings.7

Predisposing or triggering factors for Malassezia dermatitis development are as follows: increased humidity, presence of skin folds, endocrinopathies (hypothyroidism, diabetes mellitus, hyperadrenocorticism), increased populations of staphylococci, immunologic alterations (allergies, FIV, neoplasia, glucocorticoid therapy), and genetic predisposition (basset hound, West Highland white terrier, American cocker spaniel, and many other dog breeds; short-hair cat breeds such as Sphynx and Rex cats).1-3,8-12

2. Clinical signs
Predominant skin lesion in dogs are erythema, scaling, greasiness, crusts, and lichenification and hyperpigmentation (localized or generalized) in chronic cases.2 Sometimes the disease is associated with papules and even furunculosis.2 If affected, the claws show red-brown discoloration. Predilection site are lips, ear canals, groin, ventral neck, medial thighs, perianal area, interdigital skin and skin folds. In addition to skin changes, nearly all patients experience pruritus.2 In the cat, Malassezia dermatitis should be considered in following clinical presentations: otitis externa (often with accumulations of brown/black and waxy material), chin acne, facial dermatitis, paronychia and/or red-brown discoloration of the claws and generalized erythematous scaly to waxy dermatitis.2,8,11,13,14

3. Diagnosis
Main differential diagnoses include allergic dermatitis, contact dermatitis, superficial staphylococcal folliculitis, demodicosis, dermatophytosis, scabies, cheyletiellosis, feline acne and epitheliotropic lymphoma. Remember that most of the dogs and cats with Malassezia dermatitis have concurrent dermatoses or a systemic disease, which should be diagnosed appropriately.

The diagnosis is made by cytological examination using direct impressions with a microscopic slide, swab, superficial scraping or cellophane tape stripplings (for less accessible
areas such as interdigital skin or dry lesions.\(^2\) Whichever method is used, the glass slides are usually stained with Romanowsky stain (Diff-Quik®). Optionally, the samples (not scotch tape) can be heat fixated before staining.\(^15\) This may prevent loss of some material and consequently important information during the staining procedure. If one is searching for only infectious agents such as bacteria and \textit{Malassezia} just the basophilic staining component may be used. Additional utilization of the eosinophilic stain reveals important information about the inflammatory response (e.g., eosinophils). The yeasts are best visualized at 40x or 100x magnification and are round to oval shapped with or without budding (classical “peanut shape”). The yeast diameter maybe from 3-8 \textmu m in diameter.\(^2\) There is no definite yeast number to diagnose \textit{Malassezia} dermatitis. Therefore, the diagnostic criteria for \textit{Malassezia} dermatitis should always be interpreted in the clinical context. Some cases even require a therapeutical trial, to confirm the diagnosis. The fungal culture is of \textbf{no diagnostic value} in superficial infections, as dogs and cats can be healthy carriers. Important is to have typical clinical lesions with associated \textit{Malassezia} organisms found on the cytological examination.

4. Treatment

Treatment options depend on the severity of lesions and the compliance of the owner/patient. Small focal areas can be treated with antifungal creams, wipes or ear drops. In multifocal or even generalized disease, topical therapy includes application of shampoos and rinses in mono- or combined preparations: chlorhexidin, ketoconazole, miconazole, climbazole, selenium sulfide, enilconazole, lime sulfur, acetid and boric acid. When topical therapy is impractical or ineffective, oral antifungal treatment may be instituted. The most commonly used drugs are itraconazole, ketoconazole and terbinafine.\(^{13,16-21}\) As the therapeutic levels of systemic antifungals persist in the skin for several days or weeks, pulse regimens are possible. Newer treatment modalities, such as killer peptides, have been described recently.\(^22\) Very important is to consider possible drug interactions when creating a multimodal therapeutic regimen (e.g., allergic dogs treated with ciclosporine). As infections with \textit{Malassezia} sp. often co-exist with staphylococcal infections, do not forget to diagnose and treat these infections appropriately. Patients with a clinically relevant inflammatory component (usually allergic dogs), require additional anti-inflammatory drugs, such as glucocorticoids. Antifungal susceptibility testing is not routinely performed, but should be considered in refractory cases, as azole-resistant cases have been already encountered in veterinary medicine.\(^23\)

Dramatic clinical improvement is seen within 1 to 2 weeks (faster if topical and systemic medications are combined). The duration of the therapy depends on the resolution of clinical signs and should be continued 7-10 days beyond clinical cure, in average altogether 4 weeks.\(^2\) Recurrent cases of \textit{Malassezia} dermatitis are not unusual because an underlying chronic disease is often present. Frequent recurrences may require a maintenance protocol with utilization of either regular topical (once to twice weekly) or systemic antifungals (1-3 days/week pulses).
Again, to achieve a good clinical response to treatment, all efforts should be made to identify and correct the predisposing factors (allergy, hormonal disturbances, neoplasia, immune deficiencies).

References


DEMODICOSIS: TREATMENT OF DIFFICULT AND DESPERATE CASES

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1. Introduction: classic treatments

Until very recently, two treatments were considered the golden standard for canine generalized demodicosis (CGD): weekly amitraz bathing and oral macrocyclic lactones, specially ivermectin (Mueller et al, 2011).

Amitraz at 0.025% to 0.06% once a week has been demonstrated effective for treating CGD. Amitraz is a formamidine that is selective towards acarians and has been used since the late 1960s to control ticks. Amitraz does not act directly on nerve conduction. It induces an alteration of the behavior in the acarian. It interferes with the octopaminergic system of arthropods (similar to the adrenergic system in mammals) by binding to the octopamine receptors, which leads to the stimulation of monoamine oxidases (adenylate cyclase activity) and the G protein (Marc and Beugnet, 2012). It thus induces cAMP and cGMP synthesis, which have various intracellular actions. The dip should be applied carefully with a sponge and the skin saturated and allowed to air-dry without rinsing. Hair clipping is recommended in dogs with a medium or long coat. Although probably less effective than ivermectin in dogs with adult-onset disease, amitraz can be used safely in dogs with the homozygous nt228(del4) mutation of the ABCB1-1Δ gene or with adverse reactions to ivermectin. The treatment with amitraz has two problems: a cumbersome application and the side effects. Adverse effects from amitraz include depression, sleepiness, ataxia, polyphagia, polydipsia, vomiting and diarrhea. Because amitraz is an α2-adrenergic receptor agonist, atipamezole can be used to treat adverse effects.

Ivermectin is not licensed for use in canine demodicosis. However, an evidence-based review concluded that oral ivermectin at a dose of 0.3–0.6 mg/kg daily can be recommended as therapy for CGD. Considered the most effective treatment for adult-onset generalized demodicosis, ivermectin use is limited by the severe neurologic effects observed in some patients. Dogs with the ABCB1-1Δ gene defect are extremely sensitive to ivermectin and can experience severe toxicity at dosages of 100 μg/kg once a day; other mutations may have similar effects. Other canine populations (e.g., neonatal, senior, dogs on concurrent treatment with other P-glycoprotein substrates or inhibitors [spinosad,azole antifungals, erythromycin]) are also sensitive to macrocyclic lactones. This is an off label treatment, and must be administered under careful veterinary supervision and stopped immediately on detection of any clinical signs suggestive of toxicity (e.g., hypersalivation, depression, tremors, mydriasis, blindness, ataxia). Thus, a gradual dose increase from 0.05 mg/kg on day 1 to 0.1 mg/kg on day 2; 0.15 mg/kg on day 3; 0.2 mg/kg on day 4 and 0.3 mg/kg on day 5 is strongly recommended in any dog treated with ivermectin. When higher daily doses are used, a further increase by 0.1 mg/kg/day is recommended.
In both cases (amitraz or ivermectin), the treatment should be continued for 1 month after obtaining 2 consecutive negative skin scrapes 1 month apart.

2. Subcutaneous doramectin

According to a recent retrospective study (Hutt et al 2015) doramectin given by subcutaneous injection at weekly intervals is a useful and well-tolerated treatment for generalized demodicosis in the dog. Remission was achieved in 94.8% of dogs treated with weekly subcutaneous injections of doramectin at a dose rate of 0.6 mg/kg body weight. Adverse events were rare with two suspected instances (0.5%) being recorded. The mean duration of treatment was 7.1 weeks. This treatment is prescribed frequently in numerous countries. Despite the low incidence of adverse events in the study mentioned above, this treatment has a risk of neurotoxicity, similar to ivermectin. Two case reports regarding dogs exposed to doramectin give us an idea of what clinical signs can be seen. One report involved a collie given 0.2 mg/kg of doramectin SC, while the other involved 2 white Swiss shepherds exposed to 0.7 mg/kg doramectin SC. The dogs in the latter report were confirmed to have the ABCB1 gene defect, while the collie was assumed to have the gene defect. Clinical signs included blindness, restlessness, CNS depression, recumbency, hypersalivation, tremors, tachypnea, ataxia, head pressing, disorientation, lack of menace response and bradycardia. This treatment can be a good option for dogs difficult to medicate orally. However, this is not an option for dogs with sensitivity to macrocyclic lactones (due to ABCB1 gene defect or to other reasons). Eprinomectin probably has similar profile (efficacy and toxicity) to doramectin.

3. Milbemycin oxime

Milbemycin oxime, initially licensed as a heartworm preventive, is approved in some countries for treatment of demodicosis. A dosage of 1-2 mg/kg PO once a day was shown to be efficacious treatment of canine generalized demodicosis. Milbemycin is considered safe, even in dogs with the ABCB1-1Δ gene defect; however, 2 dogs with the mutation reportedly developed adverse neurologic effects with milbemycin administration. Nevertheless, milbemycin is not available in most countries as a sole agent and is very expensive if used long-term, as needed for the treatment of generalized demodicosis.

4. Topical moxidectin

Recent studies have demonstrated that topical application of 2.5% moxidectin–10% imidaclorprid is effective against canine generalized demodicosis (Paterson et al, 2014). Although oral ivermectin was shown to be more effective, weekly application of moxidectin–imidaclorprid can be an effective treatment of canine generalized demodicosis without the potential toxicity associated with ivermectin. It is also safe in dogs with the ABCB1-1Δ gene mutation. Dogs should be treated weekly and examined monthly, along with skin lesion scrapings. Treatment should be maintained, as usual, for 1 month after obtaining 2 consecutive negative skin scrapings 1 month apart, after which the product should be applied every 4 weeks to prevent relapses (anecdotal).
5. Oral isoxazolines

Isoxazolines are pesticides of a new chemical class introduced in the 2000s. They were introduced first in 2013 as veterinary products against fleas and ticks in dogs, but are also effective against numerous other external veterinary and agricultural parasites. They have a broad insecticidal and acaricidal spectrum. So far, they have been introduced only for use on dogs to prevent and treat flea and tick parasitism. Isoxazolines are non-competitive GABA (gamma-aminobutyric acid) receptor antagonists; they are much more selective for GABA receptors in insects or ticks than for those in mammals. They bind to chloride channels in nerve and muscle cells, which blocks the transmission of neuronal signals. Affected parasites are paralyzed and die. Isoxazolines approved for veterinary use so far (afoxolaner, fluralaner, sarolaner) are only for oral administration to dogs (i.e., they have a systemic mode of action). Ingested isoxazolines are rapidly absorbed into blood and distributed throughout the whole body of the host, including the skin. Blood-sucking parasites (mainly fleas and ticks) are killed during their blood meal. It is supposed that parasites living in the skin (Cheyletiella, Sarcoptes, Demodex) are also exposed to the drug and killed. Safety data collected during field studies in Europe and the USA showed that these products are well tolerated. In the field studies only mild and transient diarrhea, vomiting, lack of appetite and drooling were recorded in >2% of dogs in the first days after treatment.

Recent data (Fourie et al, 2015) suggest that fluralaner (and very likely afoxolaner and sarolaner) are effective for the treatment of generalized demodicosis. In an open study, all 8 dogs with generalized demodicosis treated with a single oral dose of fluralaner (25 mg/kg) were parasitologically negative after 56 and 84 days, and 7 of 8 exhibited hair regrowth at the end of the study (day 84). If future controlled studies confirm efficacy and safety, these molecules will become, no doubt, the standard treatment for CGD. However, because no other controlled nor larger studies are available at this time, this drug should be considered only as an alternative to the other acaricidal treatments with more evidence of efficacy (e.g., amitraz, moxidectin–imidacloprid, ivermectin).

6. Best options in challenging cases

The most adequate treatment options for the following cases of canine generalized demodicosis will be discussed with the audience:

A. Shetland sheepdog, 8 month old, tested positive for the ABCB1-1Δ gene defect.

B. Golden retriever, 13 year old, on treatment with phenobarbital (epilepsy) and gabapentin (pain –arthritis). Tested negative for the ABCB1-1Δ gene defect, but showed signs of neurotoxicity hen treated with ivermectin by mouth at 400 μg/kg once a day.

C. Mixed breed dog, 14 year old. Previous treatments with topical moxidectin weekly and daily oral ivermectin (400 μg/kg/ q 24 h) have failed.

Selected References

Fleas, mosquitoes and ticks are considered important vectors for a spectrum of infectious agents that can induce disease in dogs. The expanding number of known tick-borne organisms, the broad geographic distribution of many tick species, the ability of tick-borne organisms to induce chronic intravascular infections, and the highly pathogenic potential of some tick-borne organisms makes tick-borne infections the most important subset of canine vector-borne infectious diseases in North America and throughout much of the world. Several factors, including the ongoing Lyme Disease epidemics in the US and Europe, suburbanization of tick habitat, the rapid increase in deer numbers as well as other wildlife populations that reside within the peri-domestic environment, the recognition that same species and strains of tick-borne pathogens can induce disease in pets and their owners, and the widespread availability of safe and effective acaricides have all contributed to enhanced awareness of tick-borne infections among both professionals and non-professionals. In conjunction with the above factors, there has also been a concurrent discovery of new tick-borne organisms, for which clinical, epidemiological and pathological data is minimal or lacking, particularly in regard to disease causation in animals. Examples include *Borrelia lonestari*, *Borrelia turicatae*, *Ehrlichia muris*, Panola Mountain Ehrlichia, *Neoehrlichia mikurensis*, *Rickettsia felis*, *Rickettsia amblyommi* and many other recently described organisms for which the medical importance remains incompletely understood.

For over two decades, our research group has contributed to the development of diagnostic, therapeutic and preventive strategies for the management of infections caused by tick-transmitted intracellular organisms. As a result of these research efforts, and those of many other investigators throughout the world, we continue to gain an increasingly unique perspective on the clinical and immunopathological consequences of tick-borne infectious diseases. It has been stated that: “Ticks are only interested in nutrition (a blood meal) and sex (i.e. perpetuation of the species).” Although the tick might object to this simplistic view of its complex lifestyle, bacteria, protozoa and viruses have used the predictable behavior of all tick species to facilitate their transmission and therefore the perpetuation of their species. Transmission of a tick-borne organism is most frequently accomplished when the tick obtains a blood meal; however, transmission can occur when the tick is inadvertently ingested by a dog (*Hepatozoon canis* or *Hepatozoon americanum*). In those instances, when tick-transmission is the sole means by which an organism such as *Ehrlichia canis* is transmitted from one infected dog to a previously non-infected dog, and as the dog is the only known reservoir host for *E. canis*, it becomes obvious that *E. canis* would evolve to be efficiently transmitted by a tick (*R. sanguineus*) for which all three tick life cycle stages (larvae, nymph and adult) preferentially involve feeding on dogs. It is equally obvious that *E. canis* would seek to induce long-lasting infection, accompanied by minimal pathogenicity to the dog (do
not destroy the home you live in) and the organism would infect a cell (the monocyte) that would facilitate transfer of *E. canis* to additional blood seeking ticks. This evolutionary arrangement benefits *E. canis*, but does not appear to benefit the dog, which can develop disease manifestations ranging from epistaxis to pancytopenia. Although it is difficult to determine the factor(s) that induce disease causation when a dog is infected with a highly adapted vector-borne organism such as *E. canis*, it is certain that sequential or simultaneous infection with another vector-borne organism can contribute to more severe hematological or immunological aberrations and a more severe course of illness. Tick-borne organisms such as *Anaplasma phagocytophilum* and *R. rickettsii* typically induce acute, potentially severe illness, whereas other organisms such as *Babesia canis*, *Babesia gibsoni*, *Bartonella vinsonii* subsp. *berkhoffii*, *Bartonella henselae* and *E. canis* can induce chronic, insidious illnesses, accompanied by longstanding intravascular infections. Recent evidence from Europe supports potential transmission of *Bartonella henselae* by *Ixodes ricinus*, whereas *R. sanguineus* is the suspected vector for transmission of *B. vinsonii* subsp. *berkhoffii*. Thus ticks most likely play an important, but as yet poorly defined role, in the transmission of *Bartonella* sp. among animals and humans.

As described briefly above, specific tick species preferentially transmit different pathogenic organisms, dogs can be sequentially or simultaneously infested with more than one tick species, and a single tick can transmit more than one organism leading to co-infection. Both the tick species and the organisms that they transmit can vary substantially within and between various geographic regions. For example, infection with *R. rickettsii*, transmitted by *Dermacentor variabilis* in the state of North Carolina, occurs much more frequently in the piedmont region (central part of the state) as compared to the eastern coastal plain or the western Appalachian mountain range. All of the above factors make the diagnosis and medical management of tick-borne infectious diseases a complex and challenging task for the practicing veterinarian. Without question, the old adage “An ounce of prevention is worth a pound of cure” is applicable to any discussion of tick-borne infectious diseases. The advent of new, safe and long-lasting acaracides that can repel and kill ticks makes the prevention of tick-borne diseases an important priority for veterinarians and pet owners throughout the world. Based upon experimental infection studies, using tick attachment models, application of acaricide products can decrease the risk of transmission of *Borrelia burgdorferi*, the cause of canine Lyme borreliosis. Additional studies are needed to define the extent to which commercially available acaricides can prevent infection with various tick-borne organisms in different parts of the world.

**Spotted Fever Group Rickettsiae and Rocky Mountain spotted fever**

Spotted fever group (SFG) rickettsiae have been described from all continents. In North America, *Rickettsia rickettsii* is the most important SFG rickettsiae, where as in Mediterranean *Rickettsia conorii* is the most important SFG rickettsiae. Both of these tick-transmitted organisms can cause serious or fatal illness in dogs and people. The SFG group includes numerous closely related species including *R. rickettsii* (the type species), *R. africae*, *R. akari*, *R. australis*, *R. conorii*, *R. felis*, *R. montana*, *R. parkeri*, *R. rhipicephali* and *R. sibirica*, although many other SFG rickettsiae have been described. The typhus group rickettsiae, which includes *Rickettsia typhi* and *Rickettsia prowazekii*, have not been
implicated as a cause of illness in dogs and experimental infection of dogs with typhus group rickettsiae in our laboratory did not result in disease. Throughout various regions of the world, spotted fever group rickettsiae are transmitted by *Amblyomma, Dermacentor, Haemaphysalis, Ixodes* and *Rhipicephalus* tick species. Regardless of the strain or species of SFG rickettsiae, these organisms generally induce an acute febrile illness secondary to endothelial cell damage, which results in vasculitis, altered vascular permeability, edema and necrosis. Although it seems likely that other SFG rickettsiae could induce disease in dogs, only *R. rickettsii* in North America and *R. conorii* in southern Europe have been documented as canine pathogens. Historically in North America, only *Dermacentor variabilis* (southern and eastern United States), and *Dermacentor andersonii* (northwestern US and Canada) were known to transmit *R. rickettsii* to dogs or human beings. A recent outbreak of RMSF in Arizona was caused by *Rhipicephalus sanguineus* (The Brown Dog Tick), a tick species that is known to transmit *R. rickettsii* in Central and South America. As discussed above, *R. sanguineus* prefers to spend all three life cycle stages (larvae, nymph and adult) on a dog. When the environment is infested with large numbers of brown dog ticks or if dogs are removed from a tick infested house, human blood becomes an acceptable, if not an attractive alternative for tick feeding. During brown dog tick infestations, *Anaplasma platys*, *Candidatus Mycoplasma haematoparvum*, *Ehrlichia canis* and *R. rickettsii* can be transmitted to dogs or human beings. In the context of morbidity, mortality and severity of disease, RMSF is the most important tick-borne infection of dogs in the Americas. Due to variation in the severity and location of vascular lesions among different patients, veterinarians should anticipate a spectrum of disease manifestations following naturally-occurring infection with *Rickettsia* spp. Much of the United States is considered endemic for the ticks (*Amblyomma americanum, Dermacentor variabilis, Dermacentor andersonii*, and *Rhipicephalus sanguineus*) that transmit *R. rickettsii*. *Rickettsia rickettsii* infection (“Rocky Mountain spotted fever”) also occurs in areas of Central and South America, where several outbreaks of fatal dog and human illness has been reported. Clinical abnormalities associated with RMSF include fever, anorexia, depression, mucopurulent ocular discharge, scleral injection, tachypnea, coughing, vomiting, diarrhea, muscle pain, neutrophilic polyarthritis, and a diverse group of neurologic signs including hyperesthesia, ataxia, vestibular signs, stupor, seizures, and coma. In some dogs weight loss is very severe, considering the short duration of illness. Poorly localizing joint, muscle and/or neurologic pain suggestive of polyarthritis, polymyositis, or meningitis may represent the only or most prominent clinical finding. Retinal hemorrhages are a consistent finding, but may be absent early in the course of the disease. Epistaxis, melena, hematuria, and petechial to ecchymotic hemorrhages in the skin occur in some dogs, but may not develop unless diagnosis and treatment are delayed for five or more days after the onset of clinical signs. Scrotal edema, hyperemia, hemorrhage, and epididymal pain are frequently observed in male dogs. Signs associated with cardiovascular collapse, oliguric renal failure or brain death can develop in the terminal stages of the disease. Gangrene affecting the distal extremities, scrotum, mammary glands, nose or lips is associated with severe vascular obstruction and can induce substantial tissue loss, necessitating reconstructive surgery. Clinical manifestations in dogs are identical in most instances to manifestations reported in human patients. From a public health perspective, the dog is an environmental sentinel for RMSF and, therefore, it is important that veterinarians recognize and accurately diagnose RMSF. Diagnostic confirmation of RMSF in
a dog allows the veterinarian to discuss the risk of *R. rickettsii* transmission in the peri-
domestic surroundings, particularly as this pathogenic rickettsiae is transmitted transovarially
among some tick species.

**Canine FELINE and Human ANAPASOMOSIS AND Ehrlichiosis**

*Anaplasma phagocytophilum*, transmitted by *Ixodes scapularis*, *Ixodes pacificus*, *Ixodes ricinus* and other *Ixodes* sp. throughout the Northern hemisphere causes an acute febrile
illness in cats, dogs, horses and humans, which is often accompanied by
thrombocytopenia. Anaplasma platys, historically only thought to infect dogs, has been
associated with infections in cats and humans. At least five *Ehrlichia* spp.; *E. canis*, *E. chaffeensis*, *E. ewingii*, *E. muris* and the Panola Mountain Ehrlichia are capable of infecting
dogs and people. In the United States, both *E. chaffeensis* and *E. ewingii* cause serious
disease manifestations in people, including meningoencephalitis, acute renal failure and acute
respiratory failure.

There have been considerable advances in defining the efficacy of various antibiotics for
treatment of canine ehrlichiosis. It is now clear that 2 weeks of doxycycline is not an
effective treatment for *E. canis* infection, whereas 4 weeks of therapy (doxycycline 5mg/kg
every 12 hours) eliminates *E. canis* in both naturally and experimentally infected dogs. The
prognosis following treatment for anaplasmosis and ehrlichiosis is generally very good.
Dramatic clinical improvement usually occurs within 24 to 48 hours after initiation of
doxycycline or tetracycline in dogs with acute phase or mild chronic ehrlichiosis; however,
periods up to a year may be necessary for complete hematological recovery in chronically
infected dogs. The long term prognosis following treatment is much more variable,
potentially related to failure to diagnose concurrent infections. Undiagnosed infection with a
*Babesia* or *Bartonella* spp. can be misinterpreted as an ineffective therapeutic response when
treating ehrlichiosis, as doxycycline is generally an ineffective treatment for babesiosis and
bartonellosis. Experimentally, enrofloxacin will suppress the clinical manifestations of *E. canis*
infection and may result in hematological improvement, but does not eliminate the
infection. Although imidocarb dipropionate has gained clinical acceptance in some endemic
regions for treating severe, chronic, or presumed refractory cases of ehrlichiosis, lack of
efficacy has been demonstrated in natural and experimentally infected cases.

**Molecular Diagnostic Testing and Vector-borne Diseases**

Because most vector-borne pathogens are difficult, if not impossible, to culture from patient
samples and because many animals achieve immunological clearance following transmission
of the organism, the use of PCR to document active infection prior to or at the time of
initiation of therapy or as an aid to document therapeutic elimination of the infection has
gained acceptance among veterinary clinicians. Although PCR is a sensitive diagnostic
modality that documents the presence of DNA of the pathogen and thereby reflects active
infection, it is important for clinicians to recognize that a negative PCR result does not rule
out a tick borne infection. PCR testing for *Anaplasma*, *Babesia*, *Cyttauxzoon* (cats) *Ehrlichia*,
hemotropic *Mycoplasma*, *Leishmania* and *Rickettsia* species is available through the: Vector-
borne Diseases Diagnostic Laboratory, NCSU-CVM Rm 462A, 1060 William Moore Dr,
Raleigh NC 27607, Phone: 919-513-8279, www.cvm.ncsu.edu/docs/ticklab.html. BAPGM
Simultaneous Infection with Multiple Vector-transmitted Pathogens
Recently, simultaneous infection with more than one tick-borne pathogen has been recognized with increasing frequency in cats, dogs and human patients. Obviously, simultaneous infection with more than one tick-transmitted pathogen has important diagnostic, therapeutic and prognostic implications for the individual patient. For the most part, the pathophysiologic consequences of co-infection in dogs with various combinations of bacteria, rickettsia and protozoa have not been characterized clinically or experimentally. Although retrospective seroepidemiologic studies suggest that dogs may experience simultaneous infection with multiple tick-borne pathogens, microbiologic (culture) or molecular (PCR) evidence of simultaneous infection in dogs is currently limited. In nature, the risk of exposure to ticks, fleas, mosquitoes and biting flies is far greater for dogs than for human beings. In addition, dogs can be infested with hundreds of ticks, and at times infestation may involve different tick species. Therefore, the unknown influences of concurrent infection with multiple tick-borne pathogens, including *Anaplasma, Ehrlichia, Rickettsia, Babesia* and *Bartonella* species, on factors such as pathophysiology, diagnosis, prognosis or therapeutic outcome could be more readily characterized in dogs. Of 27 dogs that were investigated in a kennel due to increased mortality, 25 were seroreactive to an *Ehrlichia* sp., 20 to a *Bartonella* sp., 17 to a *Babesia* sp. and 22 seroconverted to *R. rickettsii* antigen. Based upon PCR analysis, most dogs were co-infected with multiple *Ehrlichia* species, as well as a *Bartonella, Babesia* or *Rickettsia* species. Prospective evaluation of sick dogs, managed in our teaching hospital, has yielded molecular evidence of co-infection with multiple tick-transmitted pathogens. Our recent experience indicates that dogs with heavy tick exposure can be infected at a high rate with multiple, potentially zoonotic, tick-borne pathogens. It is imperative that veterinarians recommend and clients use acaricide products routinely and year around to prevent flea and tick-borne infections.

Public and Occupational Health Considerations
Due to extensive contact with a spectrum of animal species, veterinarians and other animal health workers appear to have an occupational risk of infection because of frequent exposure to *Anaplasma, Bartonella, Ehrlichia*, hemotropic *Mycoplasma* spp. and potentially other tick borne pathogens, therefore these individuals should exercise increased precautions to avoid arthropod bites, arthropod feces (i.e. fleas and lice), animal bites or scratches and direct contact with body fluids from sick animals. For example, as *Bartonella* spp. have been isolated from cat, dog or human blood, cerebrospinal fluid, joint fluid, aqueous fluid, seroma fluid and from pleural, pericardial and abdominal effusions, a substantial number of diagnostic biological samples collected on a daily basis in veterinary practices could contain viable vector borne bacteria. The increasing number of defined flea and tick borne pathogens, in conjunction with the high level of bacteremia found in reservoir-adapted hosts, which represent the veterinary patient population, ensures that all veterinary professionals will experience frequent and repeated exposure to animals harboring these bacteria.
Therefore, personal protective equipment, frequent hand washing and avoiding cuts and needle sticks have become more important as our knowledge of this genus has improved and various modes of transmission have been defined.

Physicians should be educated as to the large number of tick borne pathogens in nature, the extensive spectrum of animal reservoir hosts, the diversity of confirmed and potential arthropod vectors, current limitations associated with diagnosis and treatment efficacy, and the ecological and evolving medical complexity of these highly evolved intravascular and endotheliotropic bacteria.

Selected References


A HIDDEN THREAT? FIVE KEY FACTS EVERY VETERINARIAN NEEDS TO KNOW ABOUT FELINE VECTOR-BORNE DISEASES

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Fact #1: To a substantial degree, the potential importance of several vector-borne infectious diseases in cats has not been extensively studied by veterinary clinicians or academic researchers. Throughout the world, fleas, mosquitoes, and ticks are considered the most important vectors for transmission of a spectrum of infectious agents that can induce disease in dogs; however, with the exceptions of Dirofilaria immitis (feline heartworm disease), Cytauxzoon felis (feline cytauxzoonosis), and Bartonella henselae (feline bartonellosis), known canine flea- or tick-borne pathogens have not been detected or have been minimally studied in cats. As feline heartworm disease and cytauxzoonosis are well-recognized feline vector-borne infections, these diseases will not be covered to any extent in this lecture. New information relative to the pathogenicity of Bartonella spp in cats will be presented.

Fact #2: Veterinarians associate tick-borne diseases with dogs, but not cats. This is just plain wrong. Failure to consider tick-borne infections among the diagnostic differentials for cats contributes to a failure to test for infections with vector-borne pathogens. This leads to misdiagnosis, treatment failures, and, ultimately, an ongoing lack of evidence-based literature relative to the role of tick-borne pathogens as potential causes of feline infectious diseases. Similar to dogs, cats can be infected with several tick-borne pathogens, including Anaplasma platys, Anaplasma phagocytophilum, Babesia spp, Borrelia burgdorferi, Cytauxzoon felis, and Ehrlichia spp. To date, infection with C. felis, an often fatal tick-borne infection of domestic felids, has not been reported in dogs or humans. Tick-transmitted pathogens have highly variable transmission times varying from as little as 4 hours to as long as 24- to 48-hours after tick attachment to the host for successful transmission of infectious organisms. Although laboratory data is lacking, clinical data support a rapid transmission of C. felis, as cats have died despite owners claiming routine use of historically available acaricidal products. In a recent laboratory study, the use of the imidacloprid 10%/flumethrin 4.5% polymer matrix collar (Seresto®, Bayer) prevented Amblyomma americanum transmission of C. felis to cats.¹ Thus, there is a commercially available product that may prevent transmission of the most lethal tick-borne pathogen affecting cats in North America.

Feline Anaplasmosis
Recent molecular evidence indicates that cats in North America and Europe can be infected with A. phagocytophilum. In addition to Dr. Michael Lappin at Colorado State University, our laboratory has amplified and sequenced A. phagocytophilum DNA from blood samples from cats residing in Ixodes scapularis–endemic regions.² We have also amplified A. phagocytophilum DNA from a small number of cats from the southeastern United States that
had hematologic abnormalities consistent with *A. phagocytophilum* infection (nonregenerative anemia, thrombocytopenia, or pancytopenia). Infection with *A. platys* was recently reported in a mildly thrombocytopenic cat from Brazil based on visualization of platelet inclusions (morulae). More recently, our research group has documented persistent *A. platys* infection in a cat with splenic plasmacytosis and monoclonal gammopathy; this cat was co-infected with 3 flea-vectored pathogens: *B. henselae*, *Bartonella koehlerae*, and "*Candidatus Mycoplasma haemominutum*.” Based on serologic evidence, *Bartonella* infection, most likely chronic in nature, can induce hyperglobulinemia in cats. Co-infection with multiple vector-borne pathogens is a well-recognized clinical scenario in dogs but has rarely been reported (or tested for) in cats.

**Feline Babesiosis**

As recently reviewed by the European Advisory Board on Cat Diseases, *Babesia* spp have been described in domestic cats from Brazil, India, Israel, France, Germany, Poland, Thailand, South Africa, Sudan, and Zimbabwe. However, to date, infection with a *Babesia* sp has not been described in domestic cats in North America. *Babesia felis* is endemic in South Africa, whereas *Babesia cati* is endemic in India. *Babesia leo* infects lions in South Africa. *Babesia* organisms that infect dogs, such as *Babesia canis canis*, a *Babesia microti*-like species; *B. canis presentii*; and *B. canis vogeli*, have been described in domestic cats from Spain and Portugal, Israel, and Thailand. A large *Babesia* sp infects Florida panthers, but the infectivity of this species for domestic cats is unknown. As *B. canis vogeli* is endemically transmitted by *Rhipicephalus sanguineus* to dogs throughout much of the United States, it seems plausible that feline infection with this protozoan pathogen is possible. As *R. sanguineus* prefers to infest dogs, host preference would make the possibility of *B. canis vogeli* transmission to cats much less likely. However, until serologic and molecular diagnostic panels are used routinely to test sick cats, as they currently are used to test dogs, the role of babesiosis as a disease of cats in North America will remain unknown.

**Feline Ehrlichiosis**

Morulae, indicative of infection with an *Ehrlichia* sp, have been described in stained blood smears obtained from cats in Brazil, France, Kenya, and the United States. Our research group described *E. canis*-like infection in young cats from the southeastern United States and eastern Canada. Based on polymerase chain reaction (PCR) amplification and DNA sequencing, the *Ehrlichia* DNA amplified from the blood of these cats was 100% similar to comparable *E. canis* DNA sequences obtained from canine *E. canis* isolates. We described these feline infections as *E. canis*-like, particularly as antibodies could not be detected in these cats by immunofluorescent antigen (IFA) testing using *E. canis* antigens. Interestingly, serum from all 3 cats contained anti-nuclear antibodies. Predominant disease manifestations in the 3 North American cats included (1) polyarthritis, accompanied by fever; (2) bone marrow hypoplasia or dysplasia, accompanied by pancytopenia; and (3) anemia and thrombocytopenia. Serologic studies have also supported an association between thrombocytopenia, hyperglobulinemia, and polyarthritis in cats with *E. canis* antibodies. *E. canis* DNA has been PCR amplified and confirmed by sequencing in domestic cats and wild felids (11/72 animals, 15%) in Brazil, providing additional supportive evidence that domestic cats can be infected with *E. canis*. More recently, we have confirmed infection with
Ehrlichia chaffeensis, Ehrlichia ewingii and E. canis in cats.7 Thus, cats can be infected with the same Ehrlichia spp. that induce morbidity and mortality in dogs and humans. Recent studies from Italy8 and Spain9 have also documented a spectrum of tick borne pathogens, including Ehrlichia and Babesia spp. in wild and domestic cats and in ticks attached to these cats.

**Fact #3: Fleas may well be contributing to more global health problems in cats, dogs, and people than is currently appreciated in human or veterinary medicine.** The common cat flea (Ctenocephalides felis), which infests cats, dogs, foxes, raccoons, opossums, coyotes, and other wild mammals, can be found throughout most tropical, subtropical, and temperate regions of the world. Ctenocephalides felis is known to carry and potentially transmit B. henselae, Bartonella clarridgeiae, B. koehlerae, Bartonella quintana, Mycoplasma hemofelis, Rickettsia felis, Wolbachia spp., and Dipylidium caninum.10 Cat fleas are a major source of zoonotic Bartonella spp infections and also transmit R. felis to people throughout the world.

**Feline Bartonellosis**
The extent to which Bartonella spp are pathogenic in cats remains to be determined. Because B. henselae bacteremia occurs in 25% to 41% of healthy cats in different regions throughout the world, the bacterium was considered to be nonpathogenic. However, recent data have documented variation in virulence among B. henselae strains. More pathogenic flea-transmitted strains are able to induce endocarditis, myocarditis, and death in cats.11,12 Also, blood culture documentation of B. henselae bacteremia was correlated with feline gingivitis/stomatitis, whereas serology was not statistically correlated.13 Because serologic testing does not differentiate between virulent and less virulent B. henselae strains, seroepidemiologic studies have generated contrasting results as to whether fever, lymphadenopathy, stomatitis, and gingivitis are caused by B. henselae. B. henselae DNA and intrathecal antibody production have been demonstrated in cats with neurologic disease. Immunosuppression associated with FeLV or FIV appears to increase the pathogenicity of B. henselae infection in cats. As reviewed by Guptill,14 cats experimentally infected with B. henselae developed fever, lymphadenopathy, mild neurologic signs, and reproductive disorders. In cats experimentally infected with B. henselae and B. clarridgeiae, gross necropsy results are unremarkable; however, histopathologic lesions can include peripheral lymph node hyperplasia, splenic follicular hyperplasia, lymphocytic cholangitis/pericholangitis, lymphocytic hepatitis, lymphoplasmacytic myocarditis, and interstitial lymphocytic nephritis.15 Collectively, these findings support antibiotic treatment in seroreactive or bacteremic cats with these disease manifestations.

The diagnosis of Bartonella infection should be confirmed by culturing the organism from blood or tissues such as lymph node or heart valve (endocarditis) or by amplifying Bartonella-specific DNA sequences from blood or tissues using PCR. The recent introduction of a liquid growth medium (Bartonella alpha Proteobacteria growth medium [BAPGM]) has facilitated the successful isolation of Bartonella spp from cat, dog, horse, human, and marine mammal blood samples. The use of BAPGM also allowed us to isolate B. vinsonii subsp. berkoffitii from a cat with osteomyelitis.16 The BAPGM diagnostic platform is available for testing animal and human patient samples from Galaxy Diagnostics (www.galaxydx.com).
Experimentally and clinically, doxycycline is not an effective antibiotic for elimination of *Bartonella* infections in cats. Currently, for treatment of sick cats with documented bacteremia, we are using doxycycline (5 mg/kg BID) and pradofloxacin (5 mg/kg BID) for 6 weeks. In two laboratory studies, the use of topical imidacloprid and moxidectin or an imidacloprid 10%/flumethrin 4.5% polymer matrix collar (Seresto®) prevented cat flea transmission of *B. henselae* to cats.

**Feline Hemoplasmosis**

In recent years, clinical information relative to canine, feline, and human hemoplasma infections has rapidly expanded. Hemotropic mycoplasmas (previously known as *Haemobartonella* or *Eperythorozoon* spp) can cause hemolytic anemia in cats, dogs, and other animal species. With the advent of diagnostic PCR assays targeting organism-specific gene sequences, infection with hemotropic *Mycoplasma* spp is now known to be a prevalent finding in healthy cats as well as in cats with anemia. Similar to *B. henselae*, these bacteria can most likely be transmitted by fleas, induce chronic intravascular infections, and potentially function as a primary factor or cofactor in disease expression (including anemia). Also similar to *B. henselae*, experimental evidence to support direct salivary transmission of *Mycoplasma* spp between cats is increasing. Similar to FeLV and FIV, aggressive interactions among cats may be an important means of hemotropic *Mycoplasma* transmission.

**Fact #4:** Routine, consistent prevention of flea and tick infestations is more important today than at any point in time in the history of veterinary medicine. As described above, there is increasing evidence to support a role for a range of flea- and tick-transmitted pathogens as causes of disease in cats. Also, as most of these pathogens are considered zoonotic, it is critically important to prevent flea and tick infestation of cats to protect not only the cat but also members of the family.

**Fact #5:** Veterinarians have a moral obligation and have taken an oath to protect animal and human health. Although not well recognized by the general public, veterinarians are a critically important component of the public health infrastructure in the context of client education, the routine prevention of flea and tick infestations, and collaborative interactions with local physicians regarding vector-borne infectious diseases of zoonotic importance. Prevention and elimination of mosquito bites and flea and tick infestations prevents vector-borne infectious diseases in pets and in their owners.

Previously, we described *B. quintana* bacteremia in a woman who was tested following the development of an infected cat bite lesion involving the hand. Two months later, the feral cat that had induced the bite wound was captured and was also shown to be *B. quintana* bacteremic. In a cumulative study involving 392 patients with occupational animal contact or extensive arthropod exposure, 31.9% were bacteremic with one or more *Bartonella* spp when blood, serum, and BAPGM enrichment culture PCR results were combined. Although this high prevalence of bacteremia is biased by testing at-risk, sick individuals, it clearly demonstrates that intravascular infection with *Bartonella* spp is much more common in immunocompetent patients than was previously suspected. By IFA testing, only 75 of 128 (58.6%) PCR-positive patients were seroreactive to a panel of 5 *Bartonella* spp test
antigens. In another study, DNA of Bartonella vinsonii subsp. berkholffii, B. henselae, or both organisms was amplified and sequenced from blood, BAPGM enrichment blood cultures, or autopsy tissues from 4 family members. Historical and microbiologic results derived from this family supported the possibility of human perinatal transmission of Bartonella spp. B. koehlerae bacteremia was documented in 8 immunocompetent patients (including 4 veterinarians) by PCR amplification and DNA sequencing either before or after BAPGM enrichment blood culture. Presenting symptoms among B. koehlerae bacteremic patients often included fatigue, insomnia, joint pain, headache, memory loss, and muscle pain. Four of these patients were also infected with B. vinsonii subsp. berkholffii genotype II. In another study involving 296 patients examined by a rheumatologist, the prevalence of antibodies against B. henselae, B. koehlerae, or B. vinsonii subsp. berkholffii was 62% and Bartonella spp bacteremia was documented in 41.1% of the selected patients. Co-infection with B. henselae, A. platys, and “Candidatus Mycoplasma hematoparvum” was recently reported in a veterinarian with a 2-year history of seizures. Historical exposure to animals and arthropod vectors also appears to put animal health professionals at risk for co-infections with B. henselae and hemotropic Mycoplasma spp. These and other research publications support an increasingly important One Health role for flea- and tick-transmitted organisms that infest domestic cats and wild animals as human pathogens.

References


CURRENT STATUS OF DIAGNOSIS AND TREATMENT OF
DERMATOPHYTOSIS IN COMPANION ANIMALS: PANEL DISCUSSION

Kimberly S. Coyner
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There are no written proceedings for this session — please use this space for your own notes.
GUIDELINES FOR METHICILLIN-RESISTANT STAPHYLOCOCCAL INFECTIONS: DIAGNOSIS, THERAPEUTIC CONSIDERATIONS, AND PREVENTION: PANEL DISCUSSION

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There are no written proceedings for this session — please use this space for your own notes.
THE GENETICIST’S PERSPECTIVE:
HOW TO INVESTIGATE A GENODERMATOSIS?
DIAGNOSTIC TESTS AVAILABLE TO THE DERMATOLOGIST

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Introduction
It has now become a routine process to perform DNA testing (genetic testing) in order to establish a specific diagnosis for many heritable diseases including genodermatoses. It is therefore essential that practicing veterinarians are familiar with this diagnostic method in order to understand its potential and also its limitations.

Required Sample Material
The currently available DNA tests in veterinary medicine analyze the normal genomic DNA of an individual, which is present in identical form in most cells of the body. Therefore, any nucleated cell type or tissue can theoretically be used to obtain the required genomic DNA for the assay. However, EDTA blood has become the sample of choice for any genetic test. EDTA blood samples can be shipped at room temperature to the diagnostic lab, where genomic DNA will be isolated from the blood leukocytes. EDTA blood will yield large amounts of pure high molecular weight DNA, which corresponds to largely intact long DNA molecules.

Other potential source materials include cheek swabs, hair samples, tissue samples (e.g. ear punches from livestock animals) and semen. Although cheek swabs and hair samples are very easy to take, they also have some disadvantages. Cheek swabs contain cells from the oral mucosa. It has to be kept in mind, that a cheek swab will yield only a limited amount of DNA and that the DNA of the patient will be contaminated with microbial DNA from the oral bacterial flora. If cheek swab samples are taken e.g. from all puppies of a litter, there is an elevated risk for cross-contamination between the samples in comparison to EDTA blood samples.

Hair samples should be pulled hairs with attached hair roots (10 – 50 hair roots per animal). Hair samples yield DNA of better quality than cheek swabs, and they can also be shipped very easily. However, hair samples also yield only a small amount of DNA. The limited amount of DNA that can be extracted from either cheek swabs or hair samples is sufficient for several diagnostic genetic tests, but it may be insufficient for research purposes, which often require larger amounts of DNA.

Genotyping Workflow in the Diagnostic Lab
After the DNA isolation, during most of the currently employed DNA tests, the diagnostic lab will determine the genotype of the patient at one single pre-determined position in the genome. One possible technical solution to obtain the desired genotype consists of a PCR
Step, which amplifies a short DNA fragment with the locus of interest, followed by Sanger sequencing, which reveals the exact DNA sequence at the relevant position. However, there are numerous alternative methods to genotype a DNA sample, and it is irrelevant to the clinician, which specific laboratory technology is used to determine the genotype.

**Format of the Report**
The diagnostic lab will provide the genotype as test result. For autosomal genes, a genotype should consist of two alleles. Some laboratories give the genotype in actual DNA bases (e.g. A/A, A/G, G/G), while others give some processed genotypes, such as e.g. (N/N, N/n, n/n). Ideally both the raw nucleotide genotypes as well as the interpreted processed genotype should be given. The report should also contain an explanation how the genotype should be interpreted. The interpretation will obviously depend on the mode of inheritance. A heterozygous animal (N/n) will be a healthy carrier in case of monogenic autosomal recessive inheritance. If the trait has a dominant mode of inheritance, then the heterozygous animal will be affected or is at high risk to develop the trait later in life.

The vast majority of traits, for which genetic testing is offered, follow a monogenic autosomal recessive inheritance. The possible test results for such traits are “clear” (N/N or homozygous wildtype), “carrier” (N/n or heterozygous), and “affected/at risk” (n/n or homozygous mutant). For congenital traits, it is correct to just state “affected” for the homozygous mutant animals. However, there are recessive traits, which develop only later in life, and then most labs report the homozygous mutant animals as “at risk”, which means that they will become eventually affected, if they don’t die due to other reasons.

The lab report should ideally contain an unambiguous description, which variant(s) have been analyzed. This is not trivial, but there is an internationally harmonized nomenclature for the unambiguous description of human genetic variants, which is also the standard for variants from domestic animals (http://www.hgvs.org/mutnomen/).

Finally, the lab report should contain a reference some information on the available evidence for the causality of the tested genetic variant (e.g. a reference to the scientific publication that reported the genetic variant, if available).

Currently, most diagnostic DNA tests interrogate the causative variants for truly **Mendelian traits**, which are exclusively controlled by a single gene. Thus, the causative variants are fully penetrant or there is 100% genotype-phenotype correlation. In the future, we will see more and more genetic test for **complex traits**. In complex traits there may be incomplete penetrance, e.g. only 70% of the animals with the “bad” genotype will develop the disease and there might be more than one gene with an influence on the trait. DNA tests for complex traits require a modified report format and the degree of genotype-phenotype correlation should be clearly indicated.

**Potential Problems**
A direct DNA test theoretically could have 100% sensitivity and 100% specificity. However, erroneous test results occasionally happen in reality. One of the most likely reasons for
incorrect test results are sample mix-ups. For example, when a dog breeder comes with a whole litter of young puppies and many individual samples are taken during a single session, there is a certain risk that samples get mixed up. Thus, it is very important that sample tubes are clearly labeled. Test order forms must be filled correctly and with all required data on the animal’s identity. The diagnostic laboratory has to ensure that the information provided on the test order form and the sample tube is correctly linked to the test result and the final report.

Another potential cause for incorrect test results are contaminations of the patient’s DNA with foreign genetic material. This can either happen, if the diagnostic lab does not follow high technical standards, but it can also happen during sampling, if e.g. cheek swabs or hair samples are not taken properly.

Finally, a DNA test provides meaningless information, if the wrong variant is tested. In this case, the diagnostic lab may actually very accurately determine the DNA sequence at a given position. However, if this DNA sequence is not correlated with the trait of interest, then the interpretation of this genotype (=the test result) will be meaningless. While the vast majority of DNA tests in veterinary medicine indeed interrogate the true causative variants for the investigated traits, there are some infamous examples of genetic tests that were prematurely commercialized and do not provide the desired information (e.g., Cattanach et al. 2015; Drögemüller et al. 2015). Fortunately, the vast majority of genetic tests indeed interrogate the correct causative variants for the respective traits.

Genodermatoses and other heritable phenotypes in purebred animals are much less heterogeneous than in humans. Thus, for many hereditary diseases we find that all affected individuals of one breed carry the same deleterious genetic variant. However, this is not an absolute rule, and it always has to be kept in mind that the currently offered genetic tests typically interrogate only a single position in the genome. Therefore, a positive test results clearly establishes the diagnosis, while a negative test result only excludes one particular genetic defect, but not other unknown variants, which may very well be located in the same gene. Negative genetic test results must be interpreted with care. If a genetic test has been validated in a particular breed, it should not be assumed that the test will also work in other breeds. Again, a positive test result is diagnostic, but a negative test result does not exclude all possible genetic defects. The key is recognizing that a negative test does not eliminate the possibility of a different genetic variant in the same gene as the etiology in a newly suspected case. In these difficult situations, it also may be valuable to consult with a veterinary geneticist, ideally the one who was involved in the identification of the first causative variant (O’Brien & Leeb, 2014).

**Databases on Available Tests**

The portfolio of available DNA tests changes constantly and there is currently no comprehensive database, which list all tests and all commercial labs. Many diagnostic labs give excellent information on the offered tests, but they obviously do have a conflict of interest as their business is based on selling these tests. Independent information can be found here:
Online Mendelian Inheritance in Animals (OMIA)
http://omia.angis.org.au/home/

Inherited diseases in dogs (IDID)
http://idid.vet.cam.ac.uk/search.php

WSAVA Testing Laboratories for DNA-Based and Other Genetic Tests
http://research.vet.upenn.edu/DNAGeneticsTestingLaboratorySearch/tabid/7620/Default.aspx

Conclusion
DNA based tests such as the currently employed single gene tests and possible new developments such as multi-locus and panel tests are an important tool in veterinary dermatology and veterinary medicine. As genomic technologies are rapidly advancing, it is likely that they will become even more relevant in the future.

While the single gene test is still the standard in diagnostics, in a research setting it is already possible as of today to sequence the entire genome of a patient in order to search for genetic defects. Thus, veterinary clinicians are encourage to contact geneticists, if they suspect a new genodermatosis. The identification of new causative variants has become more and more efficient over the last years and nowadays, it is entirely feasible to develop a new diagnostic DNA test with less than 5 affected patients and within 6 months of time.

Selected References
ADVANCES IN THE TREATMENT OF ICHTHYOSIS:
THE PURSUIT OF PATHOGENESIS-DIRECTED THERAPY

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SUCCESSFUL MANAGEMENT OF RELAPSING OTITIS

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1. Treatment of acute otitis externa
Individual bouts of otitis externa can be managed using polyvalent topical ear products with a glucocorticoid (to manage mild acute inflammation), an antibiotic, and an antifungal (for Malassezia). Manual cleaning may be necessary in cases with large amounts of debris.

2. Recurrent and chronic otitis
In these cases the otitis either doesn’t respond as expected (chronic persistent otitis) or recurs once treatment stops (recurrent otitis). It is important to not equate infection with inflammation in otitis. Nearly all ear infections involve commensal (e.g. staphylococci and Malassezia) or environmental (e.g. Pseudomonas) organisms that are opportunists. True primary pathogens are rare and the vast majority of infections are secondary to pre-existing inflammation, foreign bodies, obstruction or other primary problems.

Most owners and clinicians recognize ear infections, which are then successfully managed. However, the ongoing inflammation is often missed. This leads to a cycle of recurrent infection and chronic inflammation leading to progressive pathological changes and end-stage otitis that requires surgical intervention. The chronic inflammation makes each bout of infection harder to treat and repeated antimicrobial use may select for resistance.

3. Approach to chronic or recurrent otitis
Cases of acute otitis should be carefully examined to rule out foreign bodies, Otodectes etc., and check the tympanic membrane.

A. Clinical signs
Most cases can be clinically divided into erythroceruminous or suppurative otitis. Erythroceruminous otitis is characterized by erythema, pruritus and a ceruminous to seborrhoeic discharge. It is most commonly associated with a staphylococcal or Malassezia overgrowth. Suppurative otitis is characterized by erythema, ulceration, pain and a purulent discharge. Most cases are associated with a Pseudomonas infection. Otoscopy is important to determine the state of the ear canals, the type and amount of discharge and the integrity of the tympanic membrane - see the notes on Diagnostic Approach to Otitis Externa.

B. Underlying causes
Chronic or recurrent otitis should be carefully evaluated to identify primary, predisposing and perpetuating causes. Successful management requires that these are all treated. The goals are:
• Identify and manage the primary cause
• Correct predisposing factors (if possible)
• Remove debris and discharge
- Manage the secondary infection
- Reverse chronic pathological changes

C. Cytology
Cytology is necessary in all cases – see the notes on *Diagnostic Approach to Otitis Externa*.

D. Biofilms
Biofilms can be identified on otoscopy or cytology. Clinically, they form an adherent, thick and slimy discharge that is often dark brown or black. On cytology they appear as variably thick veil-like material that may obscure bacteria and cells. Biofilms are clinically important as they inhibit cleaning, prevent penetration of antimicrobials and provide a protected reservoir of bacteria. Also, antibiotics that require bacterial division will be less effective, as biofilm-forming bacteria are usually in a quiescent state. Biofims may also enhance the development of antimicrobial resistance, especially in Gram-negative bacteria that acquire stepwise resistance mutations to concentration-dependent antibiotics.

4. The potential impact of biofilms on antibiotic resistance

![Graph showing impact of biofilms on antibiotic resistance](image)

Biofilms generally inhibit antimicrobial penetration. In situations where this results in an abrupt drop in the antimicrobial concentration (solid line above), most bacteria will either be exposed to high or low antimicrobial concentrations. Most will therefore be eliminated or unaffected. The unaffected bacteria in the biofilm will act as a reservoir and lead to treatment failure, but the selection pressure for resistance is relatively low. However, with some
antimicrobial penetration into the biofilm and a gradual decrease in concentration some bacteria will be exposed to intermediate concentrations. This could provide a mutant selection window. This situation will lead to treatment failure and the development of resistance.

5. Bacterial culture and sensitivity testing
A. Using cytology to predict susceptibility patterns
Bacterial culture and sensitivity testing is not necessary in most cases of otitis externa and/or where topical therapy is used. Cytology can effectively identify the most likely organisms in most cases of otitis. This is particularly useful in mixed infections, where culture may identify several organisms with different susceptibility patterns.

*Malassezia* and staphylococci are straightforward to identify and a good estimate of their likely sensitivity can be made based on knowledge of local resistance patterns and previous treatment. Gram-negative bacteria are harder to differentiate on cytology alone, although *Pseudomonas* organisms are most common. Their susceptibility pattern is harder to predicate, although most first-time infections will be susceptible to aminoglycosides, polymixin B, silver sulfadiazine and fluoroquinolones. However, *Pseudomonas* readily acquires resistance and most isolates from recurrent infections will be multi-drug resistant.

B. Using bacterial culture and antimicrobial sensitivity testing
Bacterial culture and sensitivity testing can help identify the bacteria involved in the infection. This can be useful for less common organisms that are hard to differentiate on cytology, e.g. streptococci, enterococci, *E. coli*, *Klebsiella*, *Proteus* and coryneforms. Knowledge of their likely sensitivity patterns can help guide treatment choices.

C. Understanding breakpoints and resistance
The reported antimicrobial susceptibility results are less useful in otitis, especially with topical treatment. The breakpoints used to determine susceptibility or resistance assume systemic treatment. Briefly, the breakpoints are determined using pharmacokinetic data to estimate tissue levels following standard dosing. If the zone of inhibition around the antimicrobial disc or the minimum inhibitory concentration (MIC) exceeds the breakpoint, it is unlikely that the antimicrobial will attain a therapeutic concentration in the target tissue and that infection can be regarded as resistant to that antimicrobial. This does not necessarily mean that the bacteria are resistant to the antimicrobial, as sufficiently high levels may exceed the MIC.

Sensitivity data is less useful for topical drugs as concentrations in the ear canal are much higher than *in vitro* tests predict. The response to treatment is best assessed using clinical criteria and cytology. Antibiotic sensitivity data can be used to predict the efficacy of systemic drugs, although the concentration in the ear tissues is often low and high doses are needed.
6. Topical and systemic therapy

A. Choosing topical or systemic therapy

Topical therapy is preferred wherever possible. This results in high concentrations in the ear canals. Systemic antimicrobial therapy may be less effective in erythroceruminous otitis externa as bacteria are present only in the external ear canal and cerumen, there is no inflammatory discharge and penetration to the lumen is poor.

Systemic treatment may be more useful in suppurrative otitis externa and/or otitis media where there is an active inflammatory discharge with concurrent infection in the deep ear canal tissues (i.e. ‘pyoderma’ of the ear canal lining) and middle ear. Systemic treatment is indicated when the ear canal cannot be treated topically (e.g. stenosis or compliance problems or if topical adverse reactions are suspected) and in otitis media.

B. Topical antimicrobials

Topical products containing polymixin B, fusidic acid, florfenicol, gentamicin, enrofloxacin and marbofloxacin are suitable for most bacterial infections. Polymixin B and miconazole have synergistic activity against *Pseudomonas* and other Gram-negative organisms, and fusidic acid and framycetin show synergistic activity against staphylococci.

Fluoroquinolones, gentamicin and polymixin B are usually effective against *Pseudomonas*. Fusidic acid and florfenicol are effective against MRSA and MRSP. Neomycin is less potent than other aminoglycosides, although it is usually effective against Gram-positive bacteria. It is important to use an adequate volume to penetrate into the ear canals – 1ml is sufficient for most ears, but may be too much in very small animals and very large dogs may require more.

The efficacy of concentration dependant drugs (e.g. fluoroquinolones and aminoglycosides) depends on delivering concentrations of 10x MIC once daily. Time dependant drugs (penicillins and cephalosporins) require concentrations above MIC for at least 70% of the dosing interval. This is readily achieved with topical therapy, which achieves high local concentrations that probably persist in the absence of systemic metabolism. Concentrations of gentamicin were 3-15x and concentrations of miconazole were 1.2-2x the MIC\textsubscript{90} for canine otic isolates of staphylococci and *Malassezia*, respectively, 10 days after a five day course of Easotic®. Levels of florfenicol and terbinafine are at least 1000x MIC\textsubscript{90} for staphylococci and *Malassezia* for the duration of treatment with two doses of Osurnia®.

Removal of debris and purulent material greatly improves the efficacy of topical antibiotics, especially aminoglycosides and polymixin B. Products with chlorhexidine, acids, and/or alcohols are most effective against *Malassezia* and bacteria. Acidic ear cleaners may inactive some antibiotics (especially aminoglycosides and fluoroquinolones) although the ear canal has good buffering capacity and the pH rapidly returns to normal.

C. Systemic antimicrobials

Cefadroxil, cefalexin and clavulanate-potentiated amoxicillin are good first line drugs for staphylococcal infections. The efficacy of clindamycin and lincomycin may be limited by resistance. Cefovecin and cefpodoxime are appropriate if compliance and/or administration are likely to be difficult. Fluoroquinolones are normally reserved for second line use where
there is culture evidence that first line drugs would not be appropriate. However, penetration of antibiotics with a low volume of distribution into ear canal tissues can be limited. Fluoroquinolones have a high volume of distribution and penetrate well into most tissues; may have better efficacy in infections otherwise susceptible to other antibiotics.

7. Pseudomonas otitis
Pseudomonas are resistant to many antibiotics through low cell wall permeability, beta-lactamases, clavulanate-resistance and efflux pumps. They readily develop further resistance if treatment is ineffective as they have a large genome to express resistance genes and mutations and are capable of plasmid, transposon and bacteriophage transfer. Once fluoroquinolone resistance is established other anti-Pseudomonas antibiotics are indicated; these are often expensive, not licensed for animals and have to been given IV if used systemically.

<table>
<thead>
<tr>
<th>Antibiotics useful in Pseudomonas otitis</th>
<th>Dosage</th>
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<tbody>
<tr>
<td>Ciprofloxacin*</td>
<td>0.2% sol. 0.15-0.3 ml/ear q24h</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>15-20mg/kg PO q24h; Baytril Otic®; 2.5% injectable sol. diluted 1:4 with saline or Epiotic® topically q24h; 22.7mg/ml sol. 0.15-0.3 ml/ear q24h</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>5-10/kg PO q24h; Aurizon®; 1% injectable sol. diluted 1:4 with saline topically q24h; 20mg/ml sol. 0.15-0.3ml/ear q24h</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>Ofloxacin 0.3% 0.15-0.3 ml/ear q24h</td>
</tr>
<tr>
<td>Carbenicillin*</td>
<td>10-20mg/kg IV q8h</td>
</tr>
<tr>
<td>Clavulanate-ticarcillin*#</td>
<td>15-40 mg/kg IV q8h; reconstituted injectable sol. 0.15-0.3 ml/ear q12h; 160mg/ml sol. 0.15-0.3 ml/ear q12h</td>
</tr>
<tr>
<td>Ceftazidime*#</td>
<td>25-50mg/kg IV q8h; 100mg/ml 0.15-0.3 ml/ear q12h</td>
</tr>
<tr>
<td>Silver sulfadiazine¶</td>
<td>Dilute 0.1-0.5% in saline; combined with enrofloxacin in Baytril Otic®</td>
</tr>
<tr>
<td>Polymixin B</td>
<td>Topical Surolan® preparations</td>
</tr>
<tr>
<td>Amikacin*</td>
<td>10-15mg/kg SC q24h; 50mg/ml 0.15-0.3ml/ear q24h</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>5-10mg/kg SC q24h; topical Otomax® or Easotic®</td>
</tr>
<tr>
<td>Tobramycin*</td>
<td>Use eye drops or 8mg/ml injectable sol. 0.15-0.3ml/ear q24h</td>
</tr>
</tbody>
</table>

* - not licensed for animals; # reconstituted sol. stable for up to 7 days at 4°C or 1 month frozen; ¶ silver sulfadiazine shows additive activity with gentamicin and fluoroquinolones.

8. Potential toxicity of antimicrobials
Ticarcillin, polymyxin B, neomycin, tobramycin and amikacin are potentially ototoxic and should be used with care if the tympanic membrane is ruptured. Neomycin can cause contact reactions. Enrofloxacin, marbofloxacin, ceftazidime and silver sulfadiazine appear to be safe in the middle ear. There is potential for systemic toxicity with silver sulfadiazine and aminoglycosides in extensively ulcerated ears. The ototoxicity of gentamicin appears to depend on the preparation, and soluble gentamicin is safe. Systemic aminoglycosides can be nephrotoxic and renal function should be monitored. Fluoroquinolones can cause cartilage damage in dogs < 12 months old (18 months in giant breeds), neurotoxicity at high doses, and blindness in cats (especially with injectable enrofloxacin).
9. Treatment of biofilms and mucus
Biofilms can be physically broken up and removed by thorough flushing and aspiration. Topical trizEDTA and n-acetylcysteine can disrupt biofilms facilitating their removal and enhancing penetration of antimicrobials. Systemic administration of n-acetylcysteine is well tolerated and can help dissolve biofilms in the middle ear and other mucous surfaces. Systemic n-acetylcysteine and bromhexine can also liquefy mucus facilitating drainage in cases of primary secretory otitis media in dogs and feline inflammatory otitis media (polyps).

10. Triz-EDTA
TrizEDTA damages bacterial cell walls and increases antibiotic efficacy which can overcome partial resistance. It is best given 20-30 minutes before the antibiotic but can be co-administered. It is well tolerated and non-ototoxic. Tris-EDTA shows additive activity with chlorhexidine, gentamicin and fluoroquinolones. Solutions of 0.6% enrofloxacin, 0.2% marbofloxacin, 0.3% gentamicin, 0.1% amikacin, 2.8% ticarcillin and 1.7% ceftazidime in trizEDTA are effective against many multi-drug resistant bacteria including *Pseudomonas*.

11. Anti-inflammatory treatment
Reducing pruritus, swelling, exudation and tissue proliferation is a key goal of therapy, and maintenance treatment may be necessary in ongoing conditions such as atopic dermatitis. In addition, glucocorticoids (particularly dexamethasone) reverse the ototoxic effect of *Pseudomonas* infections. Systemic treatment may be necessary in cases with severe ear canal stenosis, and/or generalized inflammatory skin disease.

12. Ear wicks
Polyvinyl acetate ear wicks can be useful in certain cases. These are cut to size and inserted into the ear canal under anaesthesia, soaked with an antibiotic, trizEDTA and/or steroid solution and left for 3-10 days, applying the ear solution once daily. The wicks absorb discharge and draw the antibiotic solution into the ear canals. Steroid soaked wicks can resolve stenosis of the ear and prevent stenosis following sharp or laser surgery to remove polyps and other masses within the ear canal. They may prevent drainage from the middle ear in cases of discharging otitis media though. Ear wicks are tolerated provided that they are kept moist.

13. Treating otitis media
It is crucially important to flush out any debris from the middle ear cavity or pseudocavity formed from the invagination and extension of the tympanic membrane. This can only be done under general anaesthesia by passing a catheter into the middle ear. Otitis media may need 3-4 weeks (and possibly longer) systemic treatment, which is a problem if parenteral drugs are used. *Pseudomonas* infections, however, usually clear quickly once effective cleansing, antimicrobial treatment and control of the primary cause are established. Opinion is sharply divided on the systemic treatment of otitis media; some dermatologists always use systemic treatment, others instil antibiotics directly into the middle ear every 3-10 days (enrofloxacin, marbofloxacin, clotrimazole and miconazole appear to be safe to use in this way), some use topical therapy and some a combination of all the approaches.
14. Compliance and adherence
Poor compliance or adherence will compromise efficacy and encourage resistance. Compliance problems include under dosing, missed doses and stopping treatment early. Discussing potential problems openly and honestly with owners helps to select the most appropriate drug and dosing regime. Compliance can be improved by:

- Using long duration topical or injectable, once daily and/or palatable medication
- Using drugs that the owner is able to and wants to administer
- Convincing the owner of the importance of correct treatment
- Giving written instructions
- Demonstrating how to administer topical therapy and how to clean ears
- Using precise terminology – e.g. ‘every 12 hours’ instead of ‘twice daily’
- Good follow up and communication
- Minimising the number of different drugs or treatments
- Using analgesia to facilitate cleaning and topical medication

References


SKIN WARS EPISODE II – ATTACK OF THE STAPH CLONES

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Introduction
As the outermost layer of the mammalian body, the skin plays an important role in preventing microorganism invasion. Though the surface of the skin is frequently colonized by staphylococcal clones, the mammalian “light side of the force” (barrier function) prevents further “rallying” (colonization) and “breaching of the rampart” (invasion) by these Staph clones. For example, urocanic and pyrrolidone carboxylic acids, which are degradation products of filaggrin, are known to reduce the growth of Staph clones and inhibit the production of their “energy weapons” (virulence factors).1 Also, antimicrobial peptides produced in cutaneous epithelial cells promote the direct killing of Staph clones.2

However, when Staph clones opportunistically use the “dark side of the force” (virulence), their power can exceed the light side and become aggressive to mammalian skin. The aim of this review is to tell the battle story of the Staph clones in their attack on the “rampart” of the skin in staphylococcal skin infection. (Note: In this chapter, the term “rampart” refers to the living epidermis).

Rallying the Staph clones in front of the rampart
Before attacking the rampart of the skin, Staph clones rally in the stratum corneum (SC) by adherence of staphylococcal cell-wall-anchored (CWA) proteins to components of the corneocytes, or to the extracellular matrix (ECM) that can be exposed on barrier-disrupted skin. Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) such as clumping factor (Clf) and fibronectin-binding protein (Fnbp), as well as the near-iron transporter (NEAT) domains such as iron-regulated surface proteins (Isd), are the major families of staphylococcal CWA proteins.2 Previous studies found that ClfB and IsdA promote the adhesion of S. aureus to corneocytes in nasal epithelia.4, 5 S. aureus ClfB and IsdA have been reported to bind to keratin 10 (K10),4, 5 which is a cytoskeleton protein exposed on the corneocyte surface, as well as to the cornified envelope protein loricrin.4, 6 Other studies demonstrated that CWA proteins expressed in S. pseudintermedius called Sps can adhere to K10 as well as to canine corneocytes in vitro.7

Once the Staph clones have gathered in the SC, they can cooperate to attack the rampart of the skin using a quorum-sensing system - a bacterial cell–cell communication process.8 Staphylococcal accessory gene regulator D (AgrD), an oligopeptide that is further transformed to autoinducing peptide (AIP) in the environment, plays a key role in this system. When the population density of the microbes increases, AIP binds to the surface ligand on the microbes and leads to further production and derepression of virulence factors. The quorum-sensing system also contributes to development of the biofilm,9 which can protect the Staph clones from antibiotic assault. While the quorum-sensing system in S.
*aureus* has been well studied, the presence of *agrD genes*, the “operation command” for the Staph clones, in the *S. intermedius* group has also been reported in previous studies.\(^{10,11}\)

**Do the Staph clones possess “battering rams” to destroy the rampart?**

It is known that staphylococci produce a number of virulence factors, which can be considered the “energy weapons” of the Staph clones, facilitating bacterial invasion of the host organs. However, the offensive capabilities of those factors in the skin have not been fully evaluated. Previous study demonstrated that β-hemolysin exhibits a high affinity for sphingomyelin, a precursor lipid constituent of the SC ceramides, and may have sphingomyelinase activity.\(^{12}\) This weapon may help the Staph clones to penetrate to the deeper SC, but is not enough to facilitate their invasion of the living epidermis.

Exfoliative toxin (ET) is the most characterized virulence factor, capable of destroying the “mortar” in the rampart of the skin. Previous studies demonstrated that ETs produced by *S. aureus*, *S. hyicus* and *S. pseudintermedius* target desmoglein 1 (Dsg1), a desmosomal cell-cell adhesion molecule, at least in part in a species-specific manner.\(^{13-15}\) ETs dissociate the epidermal keratinocytes when injected subcutaneously in mammalian skin.\(^{13-15}\) However, a recent study demonstrated that ETs cannot penetrate tight junctions, the occluding junctions located in the upper stratum spinosum.\(^{16}\) This finding indicates that ET alone could not function as a “battering ram” to break through the gate of the rampart, and may require “covering fire” from other forces to destroy the rampart of the skin.

Staph clones breach the rampart during the counterattack of the neutrophils

Although ET alone cannot achieve the initial destruction of the rampart, ET-producing staphylococci in intraepidermal pustules are always recognized in actual cases of human and canine impetigo. In addition, cytological examination of pus from intact pustules in cases of impetigo reveals cocci-containing neutrophils. These findings raise the question of how the Staph clones interact with neutrophils, “the defense soldiers” of the host species, during the battle of cutaneous infection.

To answer this question, we recently investigated histopathological changes over time in the lesions of artificially created impetigo by inoculating mouse skin with *et* gene-harboring *S. aureus*. We found that some of the staphylococci invaded the intraepidermal clefts, but that the intrusion was recognized when neutrophils infiltrated the epidermis. Remarkably, staphylococcal intrusion into the epidermis was no longer recognized in mice with depleted neutrophils from cyclophosphamide injection. The intrusion was also recognized when mouse skin was inoculated with non-*et* gene-harboring *S. aureus*.

These findings indicate that Staph clones penetrate the rampart through the gates opened by the neutrophil troops. This gate opening might facilitate the neutrophils’ counterattack against the Staph clones, but unfortunately allows the Staph clones to sneak through at the same time. The exact molecular mechanisms by which the neutrophils gather at the epidermis and open the gate of the rampart remain to be further studied.
Exfoliative toxins act as “force lighting” to expand the battlefield

The histopathology of intraepidermal splitting recognized in impetigo indicates that ET might be “the dark side of the force” used by Staph clones to damage the rampart after the clones’ intrusion. Through studies using a mouse model of impetigo, we discovered that the intraepidermal clefts created by et gene-harboring S. aureus were significantly larger than those created by non-et gene-harboring strains. Therefore, Staph clones may use ET as “force lightning” to damage the rampart and expand the battlefield, making it more difficult for the neutrophil troops to capture the invaders for the host species.

References


SEROLOGICAL AND MOLECULAR DIAGNOSIS OF INFECTIOUS DISEASES: SOMETHING OLD AND SOMETHING NEW?

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INFECTIOUS DISEASES DIAGNOSIS: A CLINICAL EXAMPLE
I will use as an example, a consultation from a practice owner in Iowa (one of yesterday’s consults) to illustrate the complexities of infectious diseases diagnosis in the practice environment. The veterinarian contacted me by e-mail because one of his “younger associates” had evaluated a dog that initially presented for acute onset of weakness and mild anemia (PCV 27%). Following “symptomatic therapy” the hematocrit normalized for several weeks (rechecked at least 4 times), however, on a subsequent recheck, the hematocrit was 12%, immune-mediated hemolytic anemia was diagnosed, and the dog was immediately referred to Iowa State University, College of Veterinary Medicine. A tick borne pathogen panel was sent to the NCSU-VBDDL (Vector Borne Diseases Diagnostic Laboratory), but the dog died before test results were available. Babesia canis antibodies (titer 1: 64) were detected by IFA testing in our laboratory and additional review of the dog’s history determined that the dog had traveled to Arkansas. The client was very unhappy and the veterinarian was seeking information related to canine babesiosis in dogs in Iowa.

IMPLICATIONS FOR VETERINARY MEDICINE
1. A diagnosis was made (perhaps), but too late to benefit the patient.
2. Serological evidence of exposure does not confirm active infection.
3. Because of Adam Birkenheuer and others, babesiosis is an emerging canine infectious disease in the United States and throughout the world.
4. Babesiosis is a definitive cause of immune-mediated hemolytic anemia in dogs.
5. Based upon more recent observations, both visualization of Babesia organisms on blood smears and serological testing are insensitive diagnostic tests, as compared to PCR.
6. Arkansas is highly endemic for Babesia canis. Iowa is not endemic for B. canis.
7. Dogs, owners and other animals travel farther and more frequently than at any other time in history (i.e., SARS, Monkey Pox, West Nile Virus to name a few).
8. It takes only one brown dog tick to transmit this organism. This tick is usually found in kennels (boarding), homes (visiting friends) or veterinary hospitals (heaven forbid).
9. Even the best parasitology and medicine courses may not have prepared this young graduate to deal with this patient presentation. (To be sure!)
10. A complete medical history will always serve as the foundation of optimal medical care.
11. There is not a Center for Disease Control (CDC) or Infectious Diseases Society of America (or comparable organizations in other countries) that tracks companion animal infectious diseases, therefore there is no data base to address regional, national or international concerns.
SO WHAT ARE THE SOLUTIONS?
Clearly, in the context of infectious diseases, clinicians need diagnostic tests that allow for the simultaneous detection of common, uncommon and unknown infectious agents. This seemingly impossible option is becoming much more probable with the advent of molecular biology and highly sensitive and specific DNA or RNA detection systems and Next Generation Sequencing. Although molecular diagnostics have and will continue to have limitations, their incorporation into the practice of veterinary medicine is occurring at an increasingly accelerated rate. Efforts to develop highly sensitive pathogen detection methodologies for bioterrorism, will in many instances have direct applicability to clinical medicine. Bioterrorism detection has provided substantial research funding that is responsible in part for the accelerated development of technology that veterinarians will use in the future.

IMMUNOLOGICAL IMBALANCE AND INFECTION
Infection is defined as the invasion and multiplication of microorganisms in body tissues. Invasion infers that infection alters the immunological balance within an individual (i.e. invasion causes an immunological battle that can range in severity from a skirmish to all out war). It is increasingly obvious that this definition lacks clinical utility in regard to organisms that can achieve long term intracellular or extracellular persistence within the host. In these instances, it appears that both the host and the organism work in concert to maintain a state of immunological balance. For example, *Helicobacter pylori* can be found within the gastric lumen of nearly 100% of people in developing nations and 30-50% of people in developed nations. Most individuals are infected during childhood and remain infected for the remainder of their lives. Despite the large number of infected individuals and the presence of chronic superficial gastritis in all *H. pylori* infected individuals, only a small percentage of the population develops clinically apparent disease manifestations, which can include gastric ulcers, gastric MALT (mucosa associated lymphoid tissue) lymphoma or gastric adenocarcinoma. In this example, as well as others, disease expression is most likely multifactorial. It is very obvious that host genetics, microbial genetics, nutrition and exposure to toxic chemicals have a major influence on disease expression.

CONCURRENT INFECTIONS AND DISEASE EXPRESSION
If one were to assume that viruses, bacteria and protozoa had to communicate with each other in order to sustain life within a complex ecosystem, i.e. the mammalian body, then it would follow that these organisms should manipulate the highly developed and complex host immune responses in a coordinated and synergistic manner. For example, while attempting to clarify an increase in unexplained deaths in a Walker hound kennel, we were able to amplify DNA of up to six different organisms (i.e. 6 different species from 4 different genera) from an EDTA-anti-coagulated blood sample, obtained at a single point in time. What are the potential clinical implications of this observation? First, dogs with extensive tick, flea and louse exposure can be simultaneously infected (based upon detection of DNA) with multiple organisms. Secondly, these dogs were working dogs that were “genetically” capable of compensating to a substantial degree for simultaneous infection with bacteria, protozoa and rickettsiae. However, over half of the dogs in the kennel had ocular abnormalities, including anterior uveitis, ocular hemorrhage or retinitis, generally
accompanied by thrombocytopenia, mild anemia and hyperglobulinemia. Although considered by the owner’s to be functional working dogs for deer hunting, it is likely that these dogs were no longer in a state of immunological balance. It is also likely that the imbalance was multifactorial and that other factors such as nutrition and toxicity contributed to the development of ocular, hematological, cardiovascular and renal pathology. Finally, if the limited spectrum of PCR testing utilized in our laboratory was able to detect six different species in a single dog, it is highly likely that other organisms were present within this ecosystem (the blood of kennel dogs) for which no testing was performed. One possible conclusion, derived from recent research, is that the mammalian body is an ecosystem that encompasses a highly developed immune system that interacts constantly with microorganisms on the skin, mucosal surfaces and within blood and other tissues. Imbalance in the system is rarely due to a singular factor or event and rarely precipitated by a single microorganism.

**SEROLOGY AND INFECTIOUS DISEASES DIAGNOSIS**

Historically, serology has been the mainstay of diagnosis for difficult to isolate bacteria, protozoa and viruses. Serology still has an important role in infectious diseases diagnosis and ideally should be used in conjunction with isolation or molecular diagnostic approaches. Unfortunately, the isolation of many bacteria, protozoa or viruses is technically challenging, limited to research settings, and requires prolonged incubations periods (weeks to months) for successful isolation. For an acute disease process, such as Leptospirosis, Rocky Mountain spotted fever or anaplasmosis, documentation of seroconversion can be used to confirm a diagnosis. Seroconversion is defined in most instances as an at least a 4-fold increase in antibody titer between acute and convalescent test samples. Documentation of seroconversion requires a testing modality, such as indirect fluorescent antibody or kinetic ELISA that determines the quantity of antibodies in the respective patient samples. When a clinician plans to document seroconversion, an initial patient sample should be obtained as early in the course of illness as possible. As the antibody titer in the initial sample may be low (below the laboratory diagnostic cut off) or not detectable, this sample can be stored for 2-3 weeks in a refrigerator or freezer until the convalescent sample is obtained. Although seroconversion can confirm a specific diagnosis, which may be very important if the infectious agent is zoonotic, documenting seroconversion does not help the clinician in selecting a treatment approach for the patient. For acute infections, treatment such as doxycycline for acute anaplasmosis, ehrlichiosis or Rocky Mountain spotted fever, must be initiated prior to diagnostic confirmation of the disease process.

When antibodies are detected in a healthy animal or in association with a chronic infection, the presence of antibodies supports prior exposure and immunological recognition of the infecting organism, but does not confirm that the animal (healthy or sick) is actively infected with the organism for which antibodies were detected. Although treatment decisions can be, and frequently are, based upon antibody detection, this approach should be used with caution, particularly when antibodies are detected using screening tests in healthy pets. In the context of vector-borne infectious diseases, such as anaplasmosis, babesiosis, borreliosis (Lyme disease), ehrlichiosis, leishmaniasis and rickettsioses, exposure can be extensive in various animal populations. In some instances, the organism is eliminated following infection by the
host immune response, which means that active infection no longer exists and treatment directed at therapeutic elimination of the organism is not warranted. In other instances, a state of immunological balance is established between the infecting organism and the host immune response (the state of premunition). Although persistently infected, these animals can be outwardly and clinically healthy and may or may not have hematological or biochemical evidence of disease. In many instances, data is not available to define the long term consequences of these occult infections and the number of animals that will progress from a clinically healthy state to active disease. I have become somewhat fond of saying to our internal medicine residents: “We are all healthy until we get sick”. If active infection in these animals is documented by molecular testing or isolation, treatment is most likely indicated, especially if the infection can be eliminated and re-infection (through repeated tick or flea exposure) can be prevented.

Cross reactivity among various infectious agents is another limitation to serological testing. Cross reactivity among members of a genus should be an anticipated occurrence, such as the strong cross reaction between *Ehrlichia canis* and *Ehrlichia chaffeensis* or between *Anaplasma phagocytophilum* and *Anaplasma platys*. In these two examples, cross reactivity is of less clinical relevance, as in each instance both organisms with the genus respond to the same antibiotic, doxycycline. However cross reactivity between *Babesia canis* and *Babesia gibsoni* is of clinical relevance as different drugs are used to eliminate these infections. Also, for an increasing number of infections (babesiosis, bartonellosis, leishmaniasis and others) the serological response to the organism may be minimal or non-existent, despite chronic, active infection with the organism. It is important to state that all diagnostic tests have limitations that can influence the diagnostic interpretation and importantly, how a patient is treated.

When ever possible isolation, serological and molecular-based diagnostic testing should be performed in tandem. The notion that a clinician should pick a single best test or target only the most likely infectious agent in a given patient is naïve and not realistic in the clinical setting. Many infectious agents induce similar disease manifestations and it is increasingly obvious that polymicrobial infections exist more frequently than previously recognized. When confronted with a non-infectious disease process, such as cancer, thousands of dollars are spent on imaging and staging prior to the initiation of treatment. When an infectious disease is high on the list of differential diagnoses, testing dollars should be spent on determining the infectious agent or agents responsible for the disease process. Although molecular diagnostic tests have many inherent benefits, sensitivity is always an issue. Due to low template number in the test sample, a negative PCR result can never confirm that an animal is not infected with a specific pathogen. This is one of the most compelling arguments for combining serological and molecular testing modalities.

**NOVEL APPROACHES TO CULTURING BACTERIA**

In recent years, our research group has developed a novel approach to the isolation of fastidious bacteria. These efforts evolved out of our desire to isolate *Bartonella* species from dogs and people, as current microbiological approaches lack sensitivity. As a result of our development efforts, we found a liquid growth media, used to support the growth of insect
cell lines that will grow bacteria from patient samples where conventional approaches fail to achieve an isolate. Using this approach, we were able to isolate *Mycobacterium kanssii* from the pleural fluid of a dog and various bacteria form “culture-negative” pericardial fluids from racehorses residing in Kentucky. The clinical utility of this isolation approach is yet to be established, but preliminary results are most encouraging. Enhanced isolation techniques in conjunction with sensitive molecular detection techniques could drastically change perceptions related to the role of infectious agents in the expression of complex diseases, particularly autoimmune or idiopathic immune-mediated diseases.

In the context of clinical medicine, it is possible that too much microbiological emphasis and excessive clinical relevance has been accorded organisms that are easy to isolate (*Staphylococcus* and *Streptococcus* species, *Escherichia coli*) from patient samples and not enough emphasis has been placed on organisms that are highly fastidious. In the future, the use of molecular based tests that can simultaneously detect multiple organisms will clarify complex interactions that influence pathogenesis, disease expression and treatment outcomes. This is truly an exciting time for infectious disease researchers, microbiologists and clinicians.

**PCR TESTING: THE GOOD, THE BAD, AND THE UGLY**

One advantage of experience (in this case, a 30-year career as an academic internist) is the eventual realization that our collective efforts to manage illness in our patients have numerous limitations. There are obvious practical limitations, such as time, money, equipment and expertise (or the lack thereof), that confront clinicians and pathologists on a daily basis. However, there are somewhat less obvious limitations that relate to the sensitivity and specificity of diagnostic testing and the failure or inability to pursue unusual or unknown pathogens in our patients. Molecular diagnostic approaches have begun to facilitate a “modern day” revolution in our understanding of the interactions of multiple infectious agents, the complexity of disease expression induced by acute or chronic infection, and the redefinition of “previously understood” diseases such as babesiosis and leishmaniasis. Although there is an increasing appreciation for the importance of co-infection or polymicrobial infection in animal and human diseases, there are considerable gaps in our current understanding of the interactions between viruses, bacteria, protozoa and fungi as interactive contributors to complex disease expression.

Benefits of Molecular Diagnostic Testing

Without question various molecular based tools have facilitated tremendous advances in diagnosis of infectious diseases. PCR amplification of organism-specific DNA sequences can be accomplished in a matter of hours and in most instances the detection of a PCR amplicon confirms active infection with a specific organism. This approach has distinct advantages over culture, which can require incubation times ranging from days, to weeks, to months depending on the organism. In most instances, approaches that provide rapid culture results select for only a few more easily grown organisms. Although serology or examination of the cell mediated immune response will remain an important component of infectious disease diagnosis, these tests identify evidence of immune recognition of a pathogen. Limitations to the interpretation of serological test results in a given patient include: failure to confirm an active infection, cross reactivity among various infectious agents and minimal or
non-existent serological response to the organism, despite chronic and active infection for an increasing number of infections (babesiosis, bartonellosis, leishmaniasis and others).

Limitations of Molecular Diagnostic Testing
The basis of all molecular diagnostic testing is a cumulative genomic data base (Gen Bank), that provides DNA sequences, the source of these sequences and other information relative to deposit of the sequence into the data base. Unfortunately, there is substantial variation in the quality of the deposited sequence and the data that is provided relative to the source of the sequence. Although by definition a molecular based diagnostic test should be 100% specific based upon the premise that a unique gene sequence is being targeted, this is not always true or feasible for technical reasons.

Cautions Regarding Molecular Diagnostic Testing
The international genome data base (Gen Bank) is available to anyone with computer access. Therefore the sequences required for the design of a molecular diagnostic test are also available to anyone. Kits can be purchased for DNA extraction and thermocyclers for PCR amplification are no longer cost prohibitive for many commercial laboratories and are now being acquired in various practice settings. Technical expertise and rigid quality control is absolutely critical for the accurate performance of molecular diagnostic testing. Many practicing veterinarians are not familiar with the strengths and limitations of this diagnostic approach, therefore assistance in the interpretation of both positive and negative test results is frequently required. There is no standardization or quality control testing among laboratories providing molecular diagnostic test results to the public. As is stated for other consumables, “Let the buyer beware!”

There are numerous other issues that complicate and challenge the current state of the art in molecular diagnostic testing. A few of these include:

1. An exceptional well designed PCR assay that will reproducibly detect a low genome copy number (1-2 copies of an organism-specific gene target in a 200ul sample extraction) may not work as efficiently or may not work at all if transferred to a new (same manufacturer) or different thermocycler (different laboratory or different thermocycler manufacturer).

2. PCR contamination is a constant fear for the Director of a molecular diagnostic laboratory. Unfortunately, despite appropriate controls, it is impossible to prove that a given PCR amplicon obtained from a patient sample is not a function of PCR contamination in the laboratory. Although a serious concern, the use of negative controls (extraction control, PCR control) in conjunction with laboratory surveillance greatly minimizes this concern. For technical reasons, the advent of real-time PCR has further minimized this concern.

3. With the use of samples such as paraffin blocks, specific protocols must be followed to avoid DNA carry over during the collection and extraction process, which has resulted in the reporting of false positive test results and data misinterpretation in the literature.
When To Use PCR Testing
1. Use PCR prior to administration of an antibiotic or antiprotozoal drug to confirm active infection (i.e. presence of DNA equals presence of the organism). Antibody tests confirm exposure to the organism and may or may not be reflective of active infection. When in doubt, store an EDTA-anti-coagulated blood sample in the refrigerator prior to administering treatments. “It is better to have a pre-treatment sample and not need it, than to need the sample and not have it”. Avoid formalin!
2. Use PCR following completion of treatment to confirm therapeutic elimination of the infection (i.e., failure to detect DNA supports treatment success). Conceptually veterinarians can think of PCR testing using the same principles associated with culturing urine. It is best to perform PCR prior to antibiotic administration or at some time point following treatment. If treatment has not eliminated the infection, waiting 2-3 weeks following treatment should allow the organism to increase in the blood to a level that can be detected by PCR.
3. Use PCR testing when the species of an infectious agent is important for determination of the appropriate type of drug to use for treatment. For example, different drugs would be used to treat *Babesia canis* and *Babesia gibsoni* infections in dogs. Species-specific PCR allows us to differentiate the infecting species.

PCR: Points to Ponder
1. Although very sensitive tests, a negative PCR result will never definitively eliminate the possibility of an infectious agent.
2. Repeated negative PCR results would strongly support therapeutic elimination of the infectious agent.
3. The use of glucocorticoids will, in most instances, increase the number of infectious particles in the blood. Therefore, corticosteroid administration, particularly at immunosuppressive doses, can enhance PCR detection of an infectious agent.
4. If the PCR test is properly designed and properly performed, a false positive result should not occur. PCR contamination (i.e. in laboratory contamination with PCR products) can result in a false positive result. However, molecular diagnostic laboratories run controls to help avoid or to detect PCR contamination. Newer PCR approaches such as real-time PCR greatly decrease the possibility of PCR contamination.
5. PCR assays performed by different laboratories can vary substantially in quality. Always know your laboratory.

Selected References


ANTIMICROBIAL ACTIVITY OF NON-ANTIBIOTIC DRUGS AUTHORIZED FOR USE IN DOGS

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1. Background
Occurrence of methicillin-resistant staphylococci in animals is a reason for concern in relation to both public and animal health. In small animal veterinary dermatology, infections caused by methicillin-resistant Staphylococcus pseudintermedius (MRSP) pose a major therapeutic challenge since some MRSP strains, such as the European epidemic clone sequence type 71 (ST71), are virtually resistant to all systemic antimicrobial products licensed for use in dogs. As it is unlikely that new antimicrobial classes active against MRSP will enter the veterinary drug market in the near future, new therapeutic strategies are needed to exploit the current antimicrobial arsenal. Combination therapy is one of the possible strategies that can be used to manage severe MRSP infections that cannot be cured by topical antiseptic treatment. Some antimicrobial combinations such as amoxicillin clavulanate and potentiated sulphonamides are widely used in human and veterinary medicine. Research is warranted to identify new combinations of drugs acting on different targets concurrently. Pharmaceutical preparations targeting eukaryotic cells and used for management of non-infectious diseases, hereafter defined as “non-antimicrobial” drugs, represent an unexplored source to potentiate existing antimicrobials. Various non-antimicrobial drugs have been shown to have in vitro antimicrobial activity but their potential use in combination with existing antimicrobial drugs has never been tested systematically on veterinary pathogens. The objective of this study was to identify synergies between antimicrobial and non-antimicrobial drugs commonly used in small animal veterinary medicine as a possible strategy to treat MRSP infections.

2. Methods
A total of 216 antimicrobial/non-antimicrobial drug combinations were studied using a clinical MRSP ST71 strain resistant to all six antimicrobials tested, which were selected to represent the five antimicrobial classes most commonly used in dogs and cats: β-lactams [ampicillin (AMP) and oxacillin (OXA)], fluoroquinolones [ciprofloxacin CIP], lincosamides [clindamycin (CLI)], tetracyclines [doxycycline (DOX)] and potentiated sulfonamides [trimethoprim/sulfamethoxazole (SXT)]. These antimicrobials were tested in combination with 36 non-antimicrobials used in small animal practice. The latter compounds were selected on the basis of data on veterinary usage of drugs in Denmark, recommendations on frequency of usage by veterinary professionals at the local university hospital, and availability of the active compounds (Table 1).
Primary screening was performed by a double disk diffusion test using antimicrobial and non-antimicrobial disks placed at close distance on the same agar plate. A clear extension of the edge of the inhibition zone of the antimicrobial disk in proximity of the non-antimicrobial disk was interpreted as a positive result. For non-antimicrobials displaying positive results in the primary screening, Minimum Inhibitory Concentrations (MICs) were determined by broth microdilution and their synergy to selected antimicrobials was assessed by two-dimensional checkerboard assays (secondary screening). The most promising drug combination was further evaluated by exposing the model strain in exponential growth phase to the two drugs alone and in combination using drug concentrations approximating the peak serum concentration ($C_{\text{max}}$) achieved in dogs after standard dosage in single drug therapy.

**Table 1.** List of non-antimicrobial drugs used in this study.

<table>
<thead>
<tr>
<th>Non-antimicrobial drug</th>
<th>Clinical use</th>
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<tr>
<td>Prednisolone sodium phosphate</td>
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<td>Ciclosporine</td>
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Table 1. List of non-antimicrobial drugs used in this study.

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<tr>
<th>Non-antimicrobial drug</th>
<th>Clinical use</th>
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3. Results.
Seven of the 36 non-antimicrobial drugs tested in the primary screening were shown to enlarge the edge of the inhibition zone of at least one antimicrobial disk: acepromazine (CLI, OXA), amitriptyline (AMP, OXA), bromhexine (OXA), clomipramine (OXA), carprofen (AMP, DOX), fluoxetine (CIP, SXT) and ketoconazole (OXA, SXT). Ketoconazole displayed the highest antibacterial activity (MIC = 32 mg/L), followed by acepromazine, clomipramine and fluoxetine (MIC = 64 mg/L), amitriptyline and carprofen (MIC = 256 mg/L) and bromhexine (MIC > 4096 mg/L). Secondary screening by checkerboard assay revealed a synergistic antimicrobial effect between carprofen and doxycycline (ΣFICI = 0.3-0.5 depending on drug concentration). Checkerboard testing of multiple MRSP strains revealed a clear association between synergy and carriage of tetK, which is a typical feature of the MRSP ST71. An increased growth inhibition was observed when MRSP ST71 were exposed to 0.5/32 mg/L of the combination DOX/carprofen, as compared to individual drug exposure.

4. Discussion
This study indicates that approximately 19% of the 36 non-antimicrobial drugs tested were able to potentiate the antibacterial activity of one or more known antimicrobials against MRSP. All seven non-antimicrobials that were found to have antimicrobial-potentiating activity have been previously reported to possess antibacterial activity. Carprofen was shown to potentiate the antimicrobial activity of DOX as it displayed synergy at drug concentrations that may be achieved during therapy in dogs. Carprofen is a non-steroidal anti-inflammatory drug (NSAID) for veterinary treatment of inflammation and pain management. The synergy between carprofen and DOX was studied in multiple MRSP strains, leading to the identification of an association between the synergistic effect of this drug combination and strains belonging to the clonal lineage ST71, which harbours the efflux pump-mediated tetracycline resistance gene tetK. In contrast, no synergy was observed for MRSP strains belonging to another epidemic clone (ST68), which harbour an unrelated tetracycline resistance gene encoding ribosomal protection of the drug target (tetM). The association between tetK and DOX/carprofen synergy was further illustrated by the analysis of strain cultures exposed to concentrations of DOX/carprofen achievable in dogs by single therapy (0.5/32 mg/L), which showed a significantly higher growth inhibition in the three strains harbouring tetK compared to the two strains containing tetM. These results suggest that DOX/carprofen synergy only occurs in strains carrying tetK. The molecular mechanisms behind the identified synergy and its linkage to tetK remain unknown.

There is an obvious rationale for investigating the use of NSAIDs in combination to DOX for some canine infections, such as skin and upper respiratory tract infections. Interestingly, DOX has earlier been reported to have anti-inflammatory effects. Thus, the combination of the two drugs might also have enhanced anti-inflammatory activity compared to single therapy. Further pharmacodynamic and pharmacokinetic studies are needed to assess the therapeutic potential of DOX/carprofen under in vivo conditions. Carprofen analogues able to establish synergy with DOX at lower concentrations could be developed to facilitate translation of the results of this study into veterinary clinical practice.
Conclusion
Carprofen is a DOX “helper drug” that restores susceptibility to DOX in DOX-resistant MRSP strains carrying tetK. This finding is of potential clinical relevance since the epidemic multidrug-resistant clone MRSP ST71 is virtually resistant to all antimicrobial drugs licensed for veterinary use and has been previously shown to carry consistently tetK as the only tetracycline resistance determinant. More research is warranted to evaluate whether this laboratory finding can be translated into clinical practice.

Acknowledgements
The work was supported by the University of Copenhagen Research Center for Control of Antibiotic Resistance (UC-Care, www.uc-care.ku.dk) and by a grant from Zoetis. We thank the European Group for Generic Veterinary Products (EGGVP) for helping us in the collection of active ingredients of non-antimicrobial drugs.
Recurrent staphylococcal skin infections arise secondary to a variety of underlying causes. Some underlying causes are straightforward to diagnose and control, and in this case resolution of the underlying disease typically results in cessation of recurrent infection. In situations where these underlying causes have been eliminated through diagnostic evaluation, difficult-to-control causes such as atopic dermatitis (AD), “atopic-like dermatitis,” or “idiopathic superficial recurrent pyoderma (IRSP)” are considered. These latter three syndromes are characterized by recurrent infections with pruritus that is at least partially controlled with antibiotics, though varying degrees of residual pruritus may remain. They are often difficult to separate clinically, as there is considerable overlap between the syndromes. Nonetheless, all include as a major feature recurrent pruritic superficial staphylococcal pyoderma, and due to the difficulty in controlling the primary disease, recurrent pyoderma is a therapeutic challenge in these patients.

Bacterins consisting of various killed preparations of staphylococcal bacteria or their components have long been advocated as a method to reduce or eliminate recurrent staphylococcal infections. In the recent era of multi-drug resistance, there is renewed interest in the potential for use of these “vaccines” as alternatives to repeated courses of antibiotics. These notes will review the role of staphylococcal bacterins in treatment and/or prevention of staphylococcal skin infections.

What Are Staphylococcal Bacterins?
Bacterins are simply inactivated suspensions of bacteria, having been killed by heating, chemical inactivation, bacteriophage lysis, or other methods. They may be adjuvanted or unadjuvanted, and mass-produced commercially, or produced by a local laboratory for individual animals, using their resident bacterial strains. Classically, they are used to stimulate an immune response against the target organism, often an increase in antibody titer such as with common canine Leptospira or Bordetella bacterin vaccines. The earliest use of staphylococcal bacterins was probably to prevent, or lessen the severity of, staphylococcal mastitis in cattle. Mastitis vaccine products such as Lysigin® and Startvac® are based on Staphylococcus aureus and are still sold worldwide. There is reasonable evidence that they are helpful for mastitis, and the body of literature supporting their use is interesting to consider when discussing canine staphylococcal pyoderma.

How do Staphylococcal Bacterins Work?
Interestingly, there is a variety of mechanisms by which staphylococcal bacterins may produce beneficial effects. Limited data is available on precisely which action(s) are most important in dogs. Possibilities include:

- Stimulating production of antibodies against the organism or its products
- Stimulation of cell-mediated immunity against the organism
• Downregulation of a “staphylococcal hypersensitivity” reaction, as a form of “staphylococcal desensitization”

Note that in these situations, the effect is expected to be “antigen specific” so that in theory, it would be ideal to use a bacterin strain as close as possible to the one infecting the host. This has been one criticism levied against current commercial products for canine use, all of which are based on human or bovine strains of *Staphylococcus aureus*. Is it possible that there are antigens unique to *S. pseudintermedius* that are not present in an *S. aureus* bacterin? This is likely the case, but whether or not this has any practical significance has yet to be shown. On the other hand, many of the beneficial effects of staphylococcal bacterins may be due to non-antigen-specific properties, such as:

• Nonspecific immunomodulation by staphylococcal components such as teichoic acid, peptidoglycan, or exotoxins
• In products where live bacteriophage is present, phage lysis of pathogenic staphylococci causing the infection

**Current Products for Canine Use**

Prior studies have demonstrated that treatment of dogs with recurrent pyoderma with vaccine-like products derived from staphylococci can be efficacious in eliminating or limiting recurrent infections. Both autogenous bacterin preparations and a commercial lysate preparation have been shown effective. In particular, a currently-licensed veterinary product, Staphylococcus Phage Lysate (SPL) has demonstrated efficacy in this regard. SPL is prepared by bacteriophage lysis of a human-derived strain of *Staphylococcus aureus*. SPL was shown to be effective in reducing the severity and occurrence of IRSP in dogs. Its mechanism of action has, in rodent models, been shown to include both antigen-specific and nonspecific effects, and effects on cell-mediated immunity as well as on antibody titers. The effects of SPL on the canine immune system are largely unstudied, but appear to be unrelated to augmentation of antistaphylococcal antibody production. Recent gene-expression microarray studies in dogs suggest that SPL may exert its effect via upregulation of interferon-gamma production. In practice, it is sometimes used for other conditions, such as recurrent deep pyoderma, or recurrent pyoderma associated with atopic dermatitis, but efficacy in these other diseases has not yet been demonstrated by controlled trial.

**Practical Use of Staphylococcal Bacterins for Canine Pyoderma**

The group of canine patients that seem to benefit most from staphylococcal bacterins are those with IRSP. These dogs have the following characteristics:

• Superficial staphylococcal pyoderma lesions, pruritic or not.
• COMPLETE clearing of all lesions and pruritus with use of antibiotic treatment ONLY. Every time the dog is treated with antibiotics only, he/she becomes normal.
• Recurrence of lesions within 1 to 6 weeks after stopping antibiotics.
• No underlying cause found with diagnostic evaluation – normal or negative to every test.
The author’s protocol for use of SPL in canine idiopathic recurrent superficial pyoderma:
1. Begin treatment with an appropriate antibiotic, based on culture and susceptibility.
2. At the same time, begin subcutaneous injections of SPL – 0.5 cc twice weekly. The owner can give these at home.
3. After 6 weeks, stop the antibiotics. At this point, the dog should be normal. Continue the injections only for another 4 to 6 weeks. Wait to see if the dog relapses.
4. If the dog relapses badly within a few weeks, the SPL will not work. There is no need to continue it for a longer period.
5. If the dog does not relapse, the SPL is working, and eventually the owner should try 0.5 cc once weekly, and if things go well after a few months even 0.5 cc every 2 weeks.
6. Some dogs may have a partial response, with perhaps a mild relapse that seems to cure by itself without the need for antibiotics; or a greatly increased interval between relapses. This is also justification for continuing SPL treatment.
7. If treatment is successful, the total length of treatment is unknown. The author recommends continuing with 0.5cc every 2 weeks for a minimum of 1 year. If the pet is doing well, the injections can be decreased to every 4 weeks, and eventually discontinued. We have observed a substantial number of dogs in which the injections can be stopped, if given for a year or more.

Practitioner experience with bacterin treatment varies. At times and in the hands of some veterinarians, bacterins have benefited many patients. However, a common comment from small animal practitioners is that “I tried bacterins, but they just didn’t seem to be very effective in my hands.” Why such a variation in experiences? Case selection and appropriate use has much to do with clinical success in bacterin therapy. Inappropriate or premature use is bound to create failures. Currently-available bacterins are indicated primarily for IRSP or chronic pyoderma, and are NOT a substitute for identifying and treating definable underlying causes of recurrence. To initially control an episode of pyoderma bacterins are meant to be used along with antibiotics or topical antimicrobial therapy, not instead of the latter. Bacterin injections typically will not cure an active pyoderma; that is the job of the antimicrobials. Bacterins have their role in maintaining remission and preventing recurrence once the pyoderma is under control. Certainly, the response to bacterin treatment is subject to variation, based on both differences in disease states (which may involve widely differing pathogenetic mechanisms) and individual patient variation. The “bottom line” for the practitioner is that bacterins do work for some patients, and are definitely worth a try in chronic or recurrent cases of canine pyoderma. It is reasonable to pursue a course of 10 weeks of treatment; if that trial yields disappointing results, continuing is probably fruitless.

For the Future
There are a great number of unknowns about staphylococcal bacterin treatment that remain to be studied. It would be helpful to know more about the mechanism of action of these products, and if there is some clinical characteristic or biomarker that might predict success. What about products that contain *S. pseudintermedius* or *S. schleiferi* strains – might they be more effective? Might such bacterins be useful for deep pyoderma? For primary treatment?
What about route of administration – is there a place for sublingual use of staphylococcal bacterins?

Finally, it is useful to reconsider whether IRSP actually exists in dogs. Many veterinary dermatologists, including this author, have noted that the diagnosis of IRSP is being made less and less often as our clinical diagnostic acumen becomes better and better. There is some discussion that, in the past, many dogs with IRSP are actually atopic dogs or dogs with “atopic-like” dermatitis. These dogs have either very mild AD, or are a subset of AD patients whose major manifestation of their atopic state is recurrent infection and pruritus that is largely controlled with antibiotics. Interestingly, it is known that administration of injections of canine interferon-gamma to dogs with AD can provide significant clinical benefit. If by using staphylococcal bacterins for IRSP, we are stimulating the dog’s own IFN-gamma production, perhaps these dogs really just have AD, and we are just indirectly treating the AD with the dog’s “own” interferon gamma! This is but one of many mysteries about recurrent pyoderma that remains to be elucidated.
DERMAL COLLAGENS: STRUCTURE AND MECHANISTIC PATHOLOGY
PART I: STRUCTURE OF DERMAL COLLAGEN FIBERS

Thierry Olivry

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and Comparative Medicine Institute, NC State University, Raleigh, NC, USA

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A DECADE’S INCREASE IN OUR UNDERSTANDING OF CANINE ATOPIC DERMATITIS: WHAT DO WE NOW KNOW?  
PART I AND PART II  

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Introduction  
In 2001, the American College of Veterinary Dermatology Task Force on Canine Atopic Dermatitis developed a detailed series of review articles covering what was then known about the pathogenesis and clinical characteristics of atopic dermatitis (AD) in dogs. In 2015, this committee (now called “The International Committee on Allergic Diseases in Animals”, or ICADA) published a six-manuscript update to those original articles.  

The recent years have provided some truly paradigm-shifting information relating to AD as a clinical phenomenon, as well as the immune mechanisms involved in the development and maintenance of the allergic response, the critical role provided by the innate immune system (and its interactions with the environment) in the development of sensitization and the potentially critical role of skin barrier function defects in the pathogenesis of canine AD. The purpose of this two-part lecture is to provide a concise summary of the most important highlights in our updated knowledge in these areas. We will begin with a discussion about new findings in our understanding of the clinical disease as a whole. We will then take a journey from the outside to the inside of the dog, pointing out important new discoveries as we go.  

Clinical characteristics of canine AD  
Clinical criteria. Over the past 30 years, several sets of clinical criteria have been proposed to facilitate the diagnosis of AD in dogs. The most recent sets of criteria were published by Favrot et al. in 2010, and were developed based upon analysis of 1096 dogs from 15 different countries. The first of these sets of criteria represents a significant improvement in the sensitivity and specificity compared to previous criteria sets. It includes: age of onset <3 years; dog living mostly indoors; glucocorticoid responsive pruritus; chronic or recurring yeast infections; affected front feet and pinnae and non-affected pinnal edges and dorsolumbar areas. Many of these characteristics (age of onset < 3 years, involvement of distal extremities and pinnae, frequent yeast dermatitis) have also been identified in other studies seeking to characterize the phenotype of canine AD.  

Breed specific phenotypes. A significant new finding in our understanding of canine AD is the discovery that significant breed-related differences in phenotype may exist. For example, certain breeds (French bulldogs, shar-pei dogs) appear to have an earlier onset of clinical signs than is generally the case. Other breeds show a tendency to have lesions in areas other than the typical “face-feet-ears” most commonly seen, including shar-pei and West Highland white terrier dogs, which demonstrate more frequent involvement of the dorsolumbar area.
and German shepherd dogs, in which generalized involvement is frequently seen. Certain clinical lesions appear to be more common in certain breeds, including urticaria in boxers, increased predisposition to pyotraumatic dermatitis in German shepherds, golden retrievers and Labrador retrievers and increased seborrhea in West Highland white terriers. Together, these breed-specific phenotypes echo the complex interactions between genetics and the development of clinical disease.

Non-dermatologic signs associated with canine AD. Dogs affected with AD can also present with non-dermatologic signs. The presence of conjunctivitis has been reported in between 20-60% of dogs with AD, while rhinitis has been reported in 6.7% of dogs. Unfortunately, accurate determination of the true prevalence of these (and possibly other) concurrent disorders may be underreported, as relatively few articles seeking to describe the phenotype of canine AD have included information regarding the presence (or absence) of signs in organ systems other than the skin.

Relationship of atopic dermatitis to adverse food reactions. Evidence suggests that dogs have a predisposition to develop clinical signs triggered by both environmental (“classic” AD) and to food antigens. Depending upon the study, between 2 and 33% of dogs with AD have been demonstrated to also have adverse food reactions (AFR), while 10-46% of dogs with AFR also appear to be affected with AD.

It has been proposed that food allergens may be able to act as triggers for clinical AD, and this condition has variously been described as “food-induced atopic dermatitis” or canine AD sensu lato (distinguished from nonfood-responsive AD, or AD sensu stricto). Support for this hypothesis comes from several areas. First, dogs with nonfood-responsive AD often have higher levels of anti-food IgE than do healthy dogs. Second, dogs with AFR may demonstrate signs clinically indistinguishable from those of “classic” AD. Third, ingestion of allergens may be able to trigger clinical symptoms in dogs sensitized to the same allergen or to cross-reacting environmental allergens. In an example of the former, increased pruritus and lesion development was documented following ingestion of Dermatophagoides farinae or Tyrophagus putrescentiae mite allergens in dogs sensitized to house dust mite allergens. The second phenomenon has been called “oral allergy syndrome”, and has been demonstrated following tomato ingestion in a dog sensitized to cross-reacting Japanese cedar extract.

 Nonetheless, it must be pointed out that canine AFR may also present with signs not typically associated with AD, such as poor response to glucocorticoids, perianal pruritus, seborrhea, “atypical” age of onset (either younger or older than “classic AD”) and gastrointestinal abnormalities such as vomiting, diarrhea or increased frequency of defecation. For this reason, although food allergens may be important triggers of AD in some patients, it is not correct to state that canine AFR and AD are necessarily the same entity.

Environmental factors play bigger roles in the pathogenesis of AD than previously suspected. One of the biggest findings over the past 10 years has been the discovery that environmental factors have the potential to strongly influence the future path of a developing immune response. These factors exert much of their effects on the cellular, sub-cellular and non-
cellular components of the innate immune system. As a result, rather than merely playing a role as effectors of an adaptive response, these cells play a critical role in “setting the stage” for the development of allergic sensitization and disease.

*Environmental microbial factors in the pathogenesis of canine AD: the hygiene hypothesis.* In humans, an extensive body of research strongly suggests a role for environmental influences on the subsequent development of allergic skin disease. Several epidemiologic studies have demonstrated an apparent decrease in the prevalence of allergic disease in children raised in rural environments (especially in the presence of livestock). A similar decrease has been demonstrated in younger children in multi-child households, which have presumably been exposed to a heavier load of “normal” childhood infections than their older siblings. Further investigations have demonstrated an inverse correlation between the development of allergic disease and the presence of high environmental levels of endotoxin, although not all studies have supported these associations. The presumed protective effect of early-life exposure to infectious diseases and microbial agents has been named “the hygiene hypothesis”.

There are few reports specifically investigating the role of environmental microbial components in the development or clinical course of canine AD. The sole study to investigate the role of hair coat and environmental endotoxin and fungal glucan levels failed to demonstrate a significant association (negative or positive) with allergy status after adjustment for patient age and sex.

Nonetheless, indirect evidence for a possible role of environmental agent exposure has been provided by several studies which investigated the potential role of geographic, lifestyle and environmental risk factors in the development of canine AD. A reduced risk for disease development was associated with rural environments, multi-animal households, and walking in forests. In addition, the feeding of a non-commercial (and presumably microbe-rich) diet during lactation was associated with a decreased risk of the subsequent development of AD in pups. In contrast, an increased risk of disease was associated with living in cities, areas with higher population density, higher rainfall, living in a shed during puppyhood and adoption at an older age.

*Proteases.* There is evidence that exposure to environmental proteases may also play a role in the development of AD. Several allergenic proteins have active proteolytic activity. This activity has been demonstrated to play a significant role in the activation of immune responses in humans and mice, and generally appears to predispose the immune system towards a pro-allergic, T-helper 2 (Th2) dominated response. The best known of these proteolytic enzymes are derived from house dust mites (HDM). In particular, the group 1 HDM allergens have been demonstrated to produce antigen-independent activation of mast cells and other cells, inhibit negative feedback from IgE by cleaving the low affinity IgE receptor CD23, and to impair the development of Th1 immunity by cleaving CD25 (the alpha component of the IL-2 receptor) on T cells and by impairing secretion of interleukin 12 (IL-12) by dendritic cells (DC). Some of these effects are mediated by activation of protease activated receptor 2 (PAR-2). In addition, the group 1 allergens have been demonstrated to increase epithelial permeability by degradation of tight junction proteins, such as occludin,
claudin and ZO-1. Although not as well studied as the group 1 proteases, group 3, 6 and 9 HDM allergens possess serine protease activity and may also increase epithelial permeability and activate cells via PAR-2.

Evidence suggests that proteolytic enzymes have the potential to play a role in the induction of allergic responses in the dog as well. Canine PAR-2 is expressed in a number of different organs, including on keratinocytes in vivo as well as on the CPEK canine keratinocyte cell line. Although overall mRNA expression of PAR-2 was not found to differ between atopic and healthy dogs, the expression of PAR-2 protein was found to be more “patchy” in atopic dogs relative to healthy dogs. Furthermore, treatment of the CPEK cell line with proteolytically active (but not inactive) Der f 1 induced the production of granulocyte-macrophage colony stimulating factor (GM-CSF), IL-8 and tumor necrosis factor alpha (TNF-α). Treatment of the same cells with either trypsin or a PAR-2 receptor agonist increased expression of TNF-α, GM-CSF, thymus and activation regulated chemokine (TARC; CCL17) and IL-8. Finally, topical application of proteolytically active (but not inactive) cowhage induced the development of pruritus, edema and erythema in canine skin. An important point is that the ability of proteolytic compounds to enhance the development of hypersensitivity is not necessarily related to their allergenicity per se—while many of these proteases are major allergens in humans, dogs are uncommonly sensitized to them.

The role of other environmental factors in the development of canine AD. A recent study evaluating oxidative stress markers in dogs found that dogs with AD had increased serum levels of malondialdehyde (formed during lipid peroxidation) relative to healthy dogs. Furthermore, gene expression analysis has demonstrated decreased expression of methionine sulfoxide reductase A (which repairs oxidative tissue damage) in the skin of dogs with AD. These findings suggest a possible association between oxidative stress and canine AD. Further, given that allergen exposure has been associated with the induction of oxidative stress markers, cell activation and cytokine release in human bone marrow derived DC, it is tempting to speculate that allergen exposure might also influence the development of oxidative stress in canine atopics.

Environmental and/or oxidative stress have been demonstrated to upregulate the expression of heat shock proteins (HSP) in canine skin. Injection of HSP60 into the skin of healthy dogs has been demonstrated to induce expression of the (typically) regulatory cytokines IL-10 and TGF-β and the Th1 cytokine subunit IL-12p35, but this effect is decreased or absent in the skin of dogs with AD. Taken together, these results suggest that canine skin subjected to oxidative stress may secrete HSP in an attempt at immune modulation, and that atopic dog skin may be refractory to this regulation.

Finally, environmental exposure to particulate matter (such as diesel exhaust particles) and tobacco smoke has also been associated with disease development or exacerbation in humans with asthma and/or atopic dermatitis. It is possible that a similar phenomenon may also be seen in dogs, as one study demonstrated an increased rate of allergic skin disease in dogs living in households with very high environmental smoke exposure, relative to dogs living without smoke exposure.
Role of skin barrier dysfunction in canine atopic dermatitis
Until recently, most authorities considered the development of AD to be an “inside-outside” event, in which an inherently abnormal immune response induced hypersensitivity to otherwise innocuous external substances. However, more recent work suggests that dysfunction of the epidermal barrier may facilitate penetration of potential allergens and microbes, increasing the likelihood of sensitization (the “outside-inside” theory). These two views are not mutually exclusive, and integration of the two into the “outside-inside-outside” theory suggests that defects in barrier function may enhance allergen exposure, which (in genetically predisposed individuals) facilitates the development of sensitization. This sensitization then induces the release of inflammatory mediators which further exacerbate skin barrier dysfunction.

Epidermal lipids in canine atopic dermatitis. The initial evidence for the presence of defects in the lipid portion of the canine atopic epidermal barrier came from an electron microscopic study of healthy and atopic dog skin. This study demonstrated decreases in the thickness and length of the stratum corneum lipid deposits in the non-lesional skin of atopic dogs relative to healthy dogs. Furthermore, disruption of epidermal lipid lamellae was also observed. A later study confirmed the presence of lipid lamellar disorganization in experimentally sensitized dogs, and also demonstrated the exacerbation of this disorganization following allergen exposure.

More recent work has focused on the specific lipid components of the epidermal barrier, which includes cholesterol, free fatty acids and ceramides. Ceramides are lipid molecules composed of a sphingosine and one fatty acid. They play a role in the structure of the cellular lipid bilayer but may also play a role in cellular signaling. Decreases in epidermal ceramide levels have been demonstrated in canine atopic skin, and these decreases are associated with increased transepidermal water loss (TEWL). A decrease in ceramides 1 (EOS) and 9 (EOP) was demonstrated in non-lesional skin of atopic dogs relative to healthy dogs, and this decrease was associated with an increase in the proportion of epidermal cholesterol. These abnormalities appear at least in part to be secondary phenomena, as the acute inflammatory response triggered by allergen application has been shown to decrease the proportion of epidermal ceramides in sensitized dogs. Furthermore, epidermal ceramide and protein levels have been demonstrated to vary in “waves” from layer to layer, suggesting that their expression may have been altered during periods of local cutaneous inflammation. Nonetheless, experimentally-sensitized atopic dogs have also been demonstrated to display decreased ceramide levels in sites distant from allergen application as well, suggesting that these abnormalities may not be solely the result of local inflammation.

Dogs with AD have also demonstrated abnormalities in the composition of other lipids. In particular, sphingosine 1 phosphate (S1P) has been demonstrated to be significantly lower in the skin of lesional atopic canine skin relative to healthy dogs. This lipid is both a structural component and a signaling molecule that may be involved in the modulation of epidermal lipid profiles. Decreased levels of S1P were also demonstrated in the plasma of atopic dogs, suggesting a systemic aberration in the synthesis and/or metabolism of this lipid.
Epidermal proteins in canine atopic dermatitis. In humans, mutations in the gene for filaggrin (FLG) are strongly (but not inevitably) associated with the development of AD. This protein is responsible for the organization of keratin filaments into tight bundles, which is important in the development of corneocyte strength and structure. In addition, FLG is degraded into small hygroscopic molecules collectively called natural moisturizing factors, which help to maintain stratum corneum hydration.

To date, there is no consistent evidence supporting the presence of a similar loss of function defect in the majority of dogs with AD, although polymorphisms in the gene have been identified in at least one geographically and breed restricted population of dogs. Regardless, decreased expression of FLG mRNA and protein has been demonstrated in a subset of dogs with spontaneous AD. In contrast, upregulation of FLG mRNA has been demonstrated in the lesional and non-lesional skin of experimentally challenged dogs as well as dogs with spontaneous AD.

The patient-environmental interface and innate system and the development of AD
Keratinocytes:
Although keratinocytes have been regarded as relatively inert components of the epidermis, it is now evident that they are both metabolically and immunologically active. Canine keratinocytes have been demonstrated to produce a number of cytokines and chemokines, including TARC. This chemokine recruits CCR4+ T cells, which have been demonstrated to preferentially release the Th2 cytokines IL-4 and IL-13. Increased expression of TARC has been demonstrated in the skin of dogs with spontaneous atopic dermatitis, as well as following epicutaneous allergen challenge or injection of cross-linking anti-IgE antibodies.

Canine keratinocytes also produce thymic stromal lymphopoietin (TSLP), which is believed to produce a Th2-promoting phenotype in cutaneous DC. Production of TSLP has been shown to be increased in canine keratinocytes following exposure to ligands for toll-like receptors (TLR) 3 and 4. As these receptors bind microbial substances (double stranded RNA and lipopolysaccharide, respectively) this response offers one possible explanation why microbial colonization and infection may exacerbate cutaneous allergic inflammation.

Canine keratinocytes have also been demonstrated to produce a number of other inflammatory mediators, including TNF-α, IL-12p35, GM-CSF, TGF-β, IL-8, CCL27, and CCL28. Expression of some of these factors is increased following exposure to proteolytic mite allergens. Taken together, these findings suggest that keratinocytes play an important role at the patient-environmental interface, and that in doing so, that they may have a powerful influence on the subsequent responses of other cells of the immune system.

Dendritic cells:
Dendritic cells have been demonstrated to play a critical role in the development of hypersensitivity. The context in which DC encounter antigen will determine their future responses. In both humans and dogs, antigen encountered under conditions of minimal to no inflammation is generally not sufficient to trigger activation and migration to local lymphoid
tissues. If antigen is presented under these conditions, T cells may become anergic or tolerant. In contrast, antigen encounter in the presence of microbial factors, proteases or pathogen associated molecular patterns (PAMPs) triggers DC activation, migration and antigen presentation, resulting in an active T cell response.

The nature of the response is influenced by the initial stimuli encountered by the DC. For example, activation of human DC by HDM-derived proteases promotes the subsequent development of a Th2-predominant response by impairing IL-12 secretion. In contrast, activation of canine DC in the presence of lipopolysaccharide increases the expression of cytokines (including IL-1β, IL-12p40 and TNF-α) favoring the promotion of a Th1 phenotype.

The local cytokine environment also influences the polarizing potential of the DC. Dendritic cells activated in the presence of TSLP have been demonstrated to strongly induce the development of a Th2 phenotype. Canine keratinocytes have been demonstrated to produce TSLP, and this production is increased by exposure to ligands for TLR 3 and 4. Activation of canine DC in the presence of TNF-α induces the production of numerous cytokines (including IL-4, IL-13 and TGF-β), also resulting in the differentiation of naïve T cells into a Th2 phenotype.

Dendritic cells may also play a role in the effector phase of the allergic response. Both canine dermal DC and epidermal Langerhans cells (LC) have been demonstrated to express surface IgE in atopic dog skin, although it remains to be seen whether cross-linking of IgE can induce cellular activation in this species, as it does in humans. Nevertheless, both DC and LC appear to be recruited to the skin following allergen challenge or IgE-mediated mast cell degranulation.

**Mast cells:**
Mast cells have long been known to mediate the effector phase of allergic inflammation via IgE-mediated degranulation but more recent work suggests that they could potentially play a role during sensitization as well. Canine mast cells have been demonstrated to produce and release a variety of inflammatory mediators, including histamine, TNF-α, eicosanoids, IL-4, IL-13, the chemokine regulated upon activation, normal T cell expressed and secreted (RANTES; CCL5) and macrophage chemotactic protein-1 (MCP-2; CCL2). Many of these mediators are thought to play roles in the development and perpetuation of allergic responses. Canine mast cells are known to express the high affinity receptor for IgE, and crosslinking of this receptor will result in degranulation and mediator release. However, canine mast cells might also be degranulated via non-IgE dependent mechanisms. Proteases can induce IgE-independent activation of mast cells in humans and mice, and epicutaneous exposure of a proteolytic house dust mite extract was associated with cutaneous mast cell degranulation even in allergen-naïve dogs.

Mast cell degranulation has been reported to increase expression of the adhesion molecules P selectin and intercellular adhesion molecule-1 (ICAM-1) on canine endothelial cells. Increased levels of P selectin have been demonstrated in the skin of dogs with AD, with
lesser increases in ICAM-1 expression. Taken together, these findings suggest that mast cell degranulation (whether IgE-mediated or not) may enhance recruitment of inflammatory cells to the skin via the upregulation of adhesion molecules. This recruitment may either facilitate the development of a novel immune response or exacerbate an existing response.

Nonetheless, there remains some controversy regarding whether canine mast cells play a significant role in the development of canine AD. One theory is that mast cells from atopic dogs may demonstrate inherent hyper-releasability, as evidenced by increased average wheal diameter following IgE-mediated degranulation, as well as by enhanced spontaneous and mitogen-induced degranulation of mast cells derived from atopic dog skin. However, not all studies have demonstrated evidence of hyper-releasability, instead demonstrating no significant differences between mast cells obtained from atopic and healthy dogs.

Another area of controversy relates to the relative number of mast cells in canine atopic skin. Investigators have variously reported increased, decreased or unchanged numbers of mast cells in the skin of dogs with AD relative to healthy dog skin. Some of these discrepancies may be related to methodologic differences between the studies, in that degranulation or exhaustion of mast cells (as might be expected in active allergic inflammation) may artifactually decrease the number of cells detected by certain immunohistochemical or enzymatic methods. Similarly, investigations have both succeeded and failed to demonstrate evidence of active proliferation of mast cells in atopic dog skin.

Adaptive immune responses in canine AD

As for the innate immune system, recent years have demonstrated that the role of the adaptive immune system is both different and more complex than it was previously thought to be. Although the central player in the immunopathogenesis of AD remains the T cell, we have learned that their responses are neither as simple nor as polarized as originally suspected.

Lymphocyte populations in canine AD. Both CD4+ and CD8+ lymphocytes are increased in lesional and non-lesional atopic dermatitis skin relative to healthy dog skin. Many of these cells express CCR4, which is the receptor for the chemokine TARC. TARC is known to be expressed by canine keratinocytes, and both mRNA and protein expression of this chemokine are increased in the skin of atopic dogs. Expression of CCR4 mRNA has also been demonstrated to be increased in atopic dog skin, where it is probably associated with lymphocytes recruited to the skin by TARC. The numbers of cutaneous lymphocytes may also be increased by epicutaneous allergen exposure in sensitized dogs, again possibly related to the production of TARC by keratinocytes.

There are also changes in circulating T cell numbers in dogs with AD. While normal dogs were demonstrated to have a CD4+ to CD8+ ratio of 1.7:1, dogs with AD had a ratio of 2.1:1, and an even more exaggerated ratio of 2.97:1 was seen in AD dogs without pyoderma. The significance of these changes is not clear, but they likely reflect an enrichment in the CD4+ population secondary to a systemic immune response. Further analysis has demonstrated that many of these circulating CD4+ cells also express CCR4, These cells show preferential transcription of the Th2 cytokines IL-4 and IL-13 and decreased transcription of the Th1
cytokines interferon gamma (IFN-γ) and IL-2 relative to CCR4− cells. Thus, an increase in the number of these cells would promote the development and maintenance of hypersensitivity at both the local and systemic level.

Regulatory T cells in canine AD. Information about the behavior of regulatory T cells (Treg) in canine atopic dermatitis is limited, in part because of the use of different marker sets to identify these cells. For example, CD25 (the alpha chain of the IL-2 receptor) has been used to designate T cells with a putative regulatory phenotype. However, this molecule is also upregulated on activated T cells. Although some authors have found that expression of high levels of CD25 (CD25high) correlates with an increased expression of the regulatory transcript and protein FoxP3, other work has not supported this finding. Expression of regulatory cytokines such as IL-10 or TGF-β may be a more reliable indicator of Treg, but these cytokines may also serve other functions. For example, IL-10 may mediate Th2 type inflammation, while TGF-β may promote the development of a Th17 response.

As a result, putative canine Treg have been varyingly defined as CD4+/CD25+, CD4+/CD25high, CD4+/FoxP3+, CD25+/FoxP3+ and CD4+/CD25+/FoxP3+. In general, most studies have demonstrated increased numbers of circulating Treg in the peripheral blood of dogs with AD. Nonetheless, some studies have failed to demonstrate a difference in the number of circulating Treg in the blood of healthy and atopic dogs. Along similar lines, the numbers of CD25+/FoxP3+ cells were not found to differ between the skin of healthy dogs and lesional or non-lesional skin of dogs with AD. In agreement with this finding, expression of mRNA for FoxP3 also did not differ between healthy, lesional and non-lesional atopic canine skin.

Cytokines, chemokines and the Th1-Th2 balance in canine atopic dermatitis. The production of certain cytokines promotes the development of a Th2 phenotype, which favors the production of IgE and the recruitment and activation of cells typically associated with hypersensitivity, such as eosinophils. However, more recent work has demonstrated that AD is characterized by the production of a broader range of cytokines than originally thought. For this reason, it is no longer correct to consider either human or canine AD to be strictly Th2 polarized immune responses. Instead, clinical disease appears to involve a balance between Th2 cytokines as well as Th1, Th17 and regulatory cytokines. It is beyond the scope of this lecture to discuss all of the changes in cytokine production that have been demonstrated in canine AD, but a summary of some of the major findings will be presented.

Although they are no longer considered to be the only players in the pathogenesis of AD, Th2-type cytokines still have an important role in the development of disease, particularly in its early phases and in acute lesions. Interleukin 4 is considered to be the “archetypical” Th2 cytokine, but expression of this factor has been variably reported in dogs with AD. A few studies have demonstrated increased expression of mRNA for IL-4 in the skin of dogs with spontaneous AD, especially in acute lesional skin. In contrast, others have failed to detect IL-4 expression at all in the skin of either healthy or atopic dogs. Similar inconsistencies have been found in experimental models of AD, in which cutaneous mRNA expression was not detected. Furthermore, both increased and decreased expressions have been demonstrated in
peripheral blood mononuclear cells (PBMC) of experimentally sensitized dogs. Taken
together, these results suggest that IL-4 may not be as significant of a mediator of atopic
disease in the dog as it is in humans and mice.

A variety of other “Th2-type” cytokines and chemokines have been associated with
spontaneous and experimental AD in dogs, including IL-13, TSLP, IL-5, RANTES, TARC
and MCP-1. In contrast to IL-4, expression of these factors is more consistently associated
with canine atopic disease. While these factors have varying functions, they collectively
promote the production of IgE, induce the recruitment and production of eosinophils, recruit
mononuclear cells and T cells, and promote the development of Th2 polarization in T cells.

Another cytokine which appears to play a significant role in canine AD is IL-31. Although
an initial study failed to detect expression of this cytokine in the skin of dogs with AD,
subsequent work demonstrated that this cytokine was produced following allergen and
mitogen stimulation of T cells derived from HDM-sensitized dogs. Recent studies using very
sensitive assay techniques have been able to detect IL-31 in the serum of sensitized but not
healthy dogs. A direct role for IL-31 in the development of pruritus has been demonstrated
by the induction of pruritus following administration to dogs, and by a reduction in pruritus
following administration of a caninized anti-IL-31 neutralizing antibody. Nonetheless, it
remains to be determined as to whether this cytokine plays a significant role in the
development of hypersensitivity (or the inflammation associated with it) or if it instead
simply has an effector function.

T-helper 1 cytokines also appear to be involved in the pathogenesis of canine AD. The
“canonical” Th1 cytokine is IFN-γ. Unlike IL-4, IFN-γ is more consistently demonstrated in
the skin of atopic dogs, particularly in more chronic lesional skin. It has also been
demonstrated following allergen challenge in experimentally sensitized dogs, but not
following injection of cross-linking anti-IgE in the skin of healthy dogs. These findings are
consistent with the observation that IFN-γ appears to play a more of a role in the chronic
stages of disease. In contrast, expression of mRNA for IFN-γ was decreased in PBMCs from
dogs with spontaneous or experimentally-induced AD relative to those from healthy dogs.

Another significant Th1 cytokine is IL-12. This cytokine is composed of two subunits: IL-
12p35 and IL-12p40. Interpretation of gene transcription data for this cytokine is
complicated for a couple of reasons. First, the individual subunits may be incorporated into
other cytokines (e.g., IL-12p40 subunit is also part of IL-23). Second, gene transcription of
the individual components does not necessarily correspond to the levels of the active protein,
as the protein is only secreted as a heterodimer. While some studies have demonstrated
increased expression of either or both of the subunits in the skin of dogs with spontaneous or
experimentally-induced AD, other studies have failed to demonstrate differences in
expression between atopic and healthy dogs.

Evaluation of regulatory cytokines is complicated by the fact that many of these cytokines
may also serve other functions. Interpretation may be particularly confusing in the context of
active inflammation, as it may be difficult to ascertain whether these multifunctional
cytokines are contributing to inflammation or are attempting and failing to control it. Expression of TGF-β has been reported to be decreased in atopic skin and following allergen challenge, although this finding has not been demonstrated consistently. Expression of IL-10 is similarly inconsistent, with increased, decreased and unchanged expression levels reported in the skin or blood of atopic dogs.

**Role of antibodies in the pathogenesis of canine atopic dermatitis**

The role of IgE in the pathogenesis of canine atopic dermatitis. The presence of allergen-specific IgE has long been considered to be the defining feature of atopic dermatitis in dogs. Indeed, the ICADA proposed the following revised definition of canine AD in 2006: “a genetically predisposed inflammatory and pruritic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens”.

There is abundant evidence to support the assertion that IgE plays an important role in the pathogenesis of canine AD. Both spontaneous and experimental sensitzations have been associated with the development of increased levels of circulating allergen-specific IgE. Cross-linking of this IgE by the intradermal injection of anti-IgE antibodies induces the development of a local inflammatory response that is grossly and microscopically similar to that induced by the injection of allergen, and which shares many features with those seen in spontaneous disease. Furthermore, epicutaneous allergen challenge of atopic dogs will induce the development of “positive” inflammatory responses only for allergens for which the dogs have specific IgE.

However, IgE levels do not always correlate with the presence of clinical disease. Elevated levels of allergen-specific IgE and/or immediate cutaneous reactivity can frequently be demonstrated in dogs without clinical evidence of AD. Indeed, the development of allergen-specific IgE in experimentally sensitized dogs is not a guarantee of a clinical response upon subsequent allergen challenge. Conversely, allergen-specific responses may be demonstrated in sensitized dogs after IgE levels have fallen below detectable limits. Disturbingly, a recent study failed to demonstrate that atopic dogs had increased odds of elevated IgE levels for any of the evaluated allergens, relative to healthy dogs.

One possible explanation for this apparent paradox is that IgE may be functionally heterogeneous. Evidence to support this theory comes from studies demonstrating the existence of distinct fractions of IgE in the serum of artificially sensitized dogs. These fractions differed in electrophoretic mobility, antigenicity and protein A binding. In addition, leucocyte histamine release in response to anti-IgE was found to be higher in cells obtained from dogs with spontaneous AD relative to those obtained from artificially sensitized dogs, despite the presence of similar levels of total and allergen-specific IgE. However, this finding does not rule out the possibility of differences in the leukocytes themselves, rather than differences in the IgE.

**Atopic-like dermatitis.** An additional factor complicating the characterization of the role of IgE in the mediation of AD is the existence of a significant number of dogs with “atopic-like
dermatitis” (ALD), a condition which has been defined as “an inflammatory and pruritic skin disease with clinical features identical to those seen in canine atopic dermatitis in which an IgE response to environmental or other allergens cannot be documented”. It has been suggested that this condition may be similar to “intrinsic atopic dermatitis” (IAD) in humans. These patients also suffer from symptoms indistinguishable from AD but lack demonstrable immediate reactivity to allergens or elevated levels of allergen-specific IgE. Despite the clinical similarities to AD, there are significant differences between the two human disorders. Patients with IAD typically do not have a familial history of allergy, are more likely to develop symptoms in adulthood, do not typically have respiratory disease and are less likely to demonstrate epidermal barrier function defects.

It remains unknown whether canine ALD truly represents a canine analogue of IAD, or if this finding simply reflects a failure to test for relevant allergens, such as “atypical” environmental, microbial or food allergens, or even autoallergens. Unfortunately, many studies fail to distinguish between patients with canine AD and ALD. In some studies, the diagnosis of canine AD was based upon clinical signs alone, whereas in others the method of diagnosis was not mentioned or was unclear. The distinction between the two disorders may appear unimportant, as AD is generally considered to be a clinical diagnosis (largely due to the variability in IgE reactivity discussed above). However, inclusion of patients with and without allergen-specific IgE or cutaneous reactivity impairs the ability to identify differences between the groups (if they exist at all) and inhibits attempts to further clarify the role of IgE and other antibodies in the pathogenesis of canine AD.

Genetic influences on the development of canine AD
Canine AD does not appear to have a simple mode of inheritance. Early breeding studies and pedigree analyses failed to demonstrate clear evidence of heritability in dogs with AD. Although some characteristics of AD (e.g. a tendency to develop high IgE levels following early life exposure to allergen) in certain lines of Beagle dogs do appear to follow a dominant role of inheritance, the development of clinical hypersensitivity is not consistently found in these dogs.

Nonetheless, the likelihood of a genetic influence in the development of canine AD has been suggested by the strong breed predisposition for the condition. Although geographic variability does exist, most studies have found golden retrievers, West Highland white terriers, boxers, French bulldogs, German shepherds and cocker spaniels to be overrepresented. Further evidence for a possible genetic component comes from a recent study of British guide dogs, in which approximately 50% of the risk of developing clinical AD could be attributed to heritability.

More sophisticated analyses of the potential heritability of canine AD have been performed using genome-wide linkage and association studies. As might be expected from a clinically complex condition, no single gene (or multiple genes) has been consistently identified in association with canine AD. In contrast to humans, multiple studies have failed to demonstrate an association between canine AD and the filaggrin (FLG) locus, although one study did demonstrate an association in UK Labrador retrievers. However, other potential
candidates have been identified. These include PTPN22 (protein tyrosine phosphatase, non-receptor type 22; involved in the prevention of spontaneous lymphocyte activation; chromosome 17); the canine orthologue of the cytochrome P450 26B1 gene (involved in adipogenesis and retinoic acid pathways; chromosome 17); RAB3C (a transporting protein found on lipid anchors; chromosome 2); the thymic stromal lymphopoietin receptor (chromosome X); PKP2 (a structural component of the desmosomes and corneodesmosomes; chromosome 27); and DLA-79 (a MHC class Ib molecule; chromosome 18). It is worth noting that some of these genes may be directly or indirectly related to epidermal barrier structure and function, suggesting that barrier features other than FLG may play a more significant role in this species than in humans.

Interestingly, analyses performed on 659 dogs from the USA, UK and Japan were able to associate polymorphisms in several genes with atopic status, but the majority of these associations were breed-specific, or were restricted to breeds within specific geographic areas. These breed-regional specificities echo the geographic variability observed in the clinical breed prevalence of AD, demonstrate the significance of regionally-associated differences in genetic backgrounds within breeds, and suggest that further investigation of intra-breed differences in susceptibility may facilitate the identification of additional factors relative to the development of clinical disease.

Summary
The past 10 to 15 years have provided a wealth of new knowledge in the subject of canine AD, but there is still very much left to learn. We have further defined the clinical phenotypes associated with canine AD, but have only begun to identify some of the interactions between genetic background and variations in clinical manifestations of disease. We have learned that environmental factors may interact with the immune system in a manner more significant than previously suspected, and that those interactions may ultimately be impacted by genetic factors regulating items as diverse as epidermal permeability and cellular activation. We now know that, rather than simply serving as effectors for the adaptive response, cells of the innate immune system (including keratinocytes) actively contribute to the allergic response and, in fact may play a critical part in the determination of whether or not a hypersensitivity is generated. New work has demonstrated that the old Th1-Th2 paradigm was greatly oversimplified, and that multiple functional populations likely contribute to clinical disease. Finally, although we have learned much about the role of IgE in canine AD, there are still many phenomena (such as ALD) which frustrate our attempts at understanding. It is to be hoped that the next decade will yield just as many exciting new pieces of information, and will lead to better methods for prevention, diagnosis and treatment of this fascinating and frequently frustrating disorder.

Selected References
(Complete reference list available upon request; cpucheu@lsu.edu)


MESENCHYMAL STEM CELL THERAPY IN VETERINARY DERMATOLOGY

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1. Introduction
Together with monoclonal antibodies, the use stem cells is one of the most important therapeutic innovations of the last years. The availability of mesenchymal stem cells (MSCs), even as a commercial product, is bringing a sense of urgency in addressing many questions regarding the indications, efficacy, safety and potential of this treatment in veterinary dermatology. To date, MSCs have been used in a wide range of human and veterinary dermatologic conditions, with some clinical trials reporting benefits and almost no toxicity.

Mesenchymal stem cells are non-haematopoietic cells that reside in most tissues including adult bone marrow. For nomenclature and types, please see the review by Harman et al 2013. The strategy to isolate these cells relies exclusively on their ability to adhere to plastic in culture and their extensive proliferative capacity in vitro. Once isolated, the cells are tested for specific surface markers. MSCs are negative for normal haematopoietic markers such as CD14, CD34 and CD45, but they are expected to display at least CD73, CD90 and CD105. The end result is a heterogeneous population, where most cells have only limited differentiation capacity and only 30–50% of colony-forming cells have the actual tri-lineage plasticity (bone, cartilage or adipose tissue). These cells can be maintained frozen to be delivered to the patient when needed.

The properties of MSCs that make them interesting as a therapeutic tool are (Khorostehrani, 2013):

A. They are able to home to areas of inflammation and tissue damage such as wounds or neoplasia.
B. They have immunosuppressive properties. They can modulate innate and adaptive immune responses. MSCs secrete several soluble factors (PGE2, NO, IDO, etc.) that together can trigger generation of regulatory T cells and anti-inflammatory M2 monocytes/macrophages. In concert with these cells, MSCs can suppress the differentiation of antigen presenting dendritic cells as well as the functions of helper T cells, B cells, NK cells, and mast cells. By secreting IL-6 MSCs can also prevent apoptosis of neutrophil granulocytes thereby supporting their antibacterial functions. For detailed information about MSCs and immunosuppression, please see the review by Nemeth and Mezey (2015).
C. They promote angiogenesis and neovascularization by the secretion of cytokines, such as hepatocyte growth factor, vascular endothelial growth factor, placental growth factor, transforming growth factor, fibroblast growth factor and angiopoietin.
D. They have differentiating capacity and can differentiate into cartilage, bone or fat, but also other mesenchymal lineages. Of special interest to dermatology, MSCs can differentiate into fibroblasts in vitro if exposed to connective tissue growth factor.
(CTGF). These properties suggest that MSCs could replace defective cells in wounds, bones, cartilages and regenerate de novo the tissue.

So far two main types of MSCs have been used in veterinary dermatology:

i. Autologous stem cells, obtained from the adipose tissue or from bone marrow of the patient.

ii. Heterologous stem cells, using established stem cell lines, from human (xenogenic) or canine origin (allogenic), in most cases of embryonic origin.

2. Use of MSCs in chronic non-healing wounds

A key area of need in cell therapy in dermatology is wound healing. Skin wound healing is a complex process necessitating the interplay between various populations of cells. It has three overlapping stages: the inflammatory phase that can lead to chronic wounds if persistent; new tissue formation that includes the recruitment of myofibroblasts, angiogenesis and re-epithelization; and finally, the remodeling phase where fibroblasts from adjacent dermis invade the scar tissue and rearrange collagen bundles. This process can be disturbed by many different causes and situations resulting in chronic non-healing wounds and in different types of hypertrophic and pathologic scars. MSCs have been used with success in wound healing because they can contribute to the pool of dermal fibroblasts and generate a new dermis. In addition, dermal mesenchymal cells have been shown to sustain the interfollicular epidermis through production of growth factors such as GM-CSF, keratinocyte growth factor (KGF) or IGF1 among others, helping to promote re-epithelization. Two areas where MSCs have demonstrated to be very effective are the treatment of chronic diabetic and decubitus ulcers (MSCs are delivered mixed with a fibrin net; Dabiri et al, 2013) and the treatment of severe skin burns (40% body surface) (Ozturk and Karagoz, 2015). There are anecdotal reports of the use of this treatment in veterinary medicine.

3. Inflammatory and immune-mediated disorders

From the above listed properties, the immunosuppressive activity of MSCs has been the most widely used in a clinical setting to treat inflammatory disorders. In human dermatology, clinical trials have established the benefit of MSCs in graft versus host disease (GVHD), fistulizing Crohn’s disease and in autoimmune disorders with skin manifestations such as systemic sclerosis, lupus erythematosus or dermatomyositis. It is therefore probable that MSCs will be soon offered in an even wider range of skin inflammatory disorders.

In veterinary medicine MSCs were used first to treat canine osteoarthritis and tendon injuries in horses. In veterinary dermatology, MSCs have been used to treat two conditions: atopic dermatitis and perianal fistulas in dogs.

Atopic dermatitis is certainly a promising area, considering both the pathophysiology of the disease and immunoregulatory properties of stem cells. However, one pioneer clinical trial reported lack of efficacy of intravenous administered MSCs to atopic dogs (Hall et al, 2010). This could however be consequence of a low dose or frequency of administration. New clinical trials are exploring the efficacy of different types of MSCs and doses in the treatment of canine atopic dermatitis.
At least three clinical trials have explored the use of stem cells in the treatment of perianal fistulas, with good results. A major advantage of this condition is that the stem cells can be injected directly in the lesion, increasing efficacy. It seems that best results have been obtained so far with a line of human embryonic mesenchymal stem cells (Ferrer et al, 2015). This cell line has a marked immunosuppressive profile and it is therefore indicated for treating autoimmune and immune mediated disorders. Similar good results have been obtained in humans treating fistulizing Crohn’s disease with intralesional injections of autologous mesenchymal stem cells.

Diseases that, according to their physiopathology, could be good candidates for MSC therapy are also canine familial dermatomyositis and ischemic dermatopathies and canine pemphigus foliaceus. In fact, most diseases treated currently with immunosuppressive drugs could be adequate candidates for stem cell therapy, if the adequate MSCs are used.

4. Future trends

A. **Better understanding of the mechanism of action of MSCs.** Many aspects of the mechanism of action of MSCs are unknown; for instance: the survival in tissues and the duration of the action and the role that play microvesicles and non-coding RNAs in the mechanism of action.

B. **Development of controlled trials to show evidence of efficacy.** So far, the treatment with stem cells have proved to be very safe, but there are still controversies about its efficacy. Well-designed and controlled clinical trials are necessary to generate evidence of efficacy.

C. Development of clinical trials to define dosing, route and frequency of administration and the use of stem cell treatment in combination with other therapies.

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ARE ANTIHISTAMINES USEFUL IN THE TREATMENT OF CANINE ATOPIC DERMATITIS?

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There are no written proceedings for this session — please use this space for your own notes.
LEISHMANIA: CHALLENGING CASES

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Introduction
Cutaneous problems represent the most common clinical manifestation of canine leishmaniosis (CanL) [canine Leishmania infantum infection] and are observed in almost all affected dogs. Skin lesions are remarkably variable, thus in endemic areas CanL is frequently included in the list of differentials of dermatological disorders. Causes of pleomorphism, which is both clinical and histopathological, are not completely understood. Nonetheless, the diversity of immune response against Leishmania likely contributes to explain the variability of clinical and histopathological features in infected dogs.

Independently from the clinical presentation, staging via clinicopathological and serological examinations should represent the first step in the diagnostic protocol of CanL. Furthermore, in case of cutaneous problems, a correlation between the cause (Leishmania) and the effect (skin lesions) should be established. This is achieved by demonstrating the presence of parasites in lesional skin through various techniques, including cytology, histopathology, immunohistochemistry (IHC) and different methods of polymerase chain reaction (PCR).

Leishmania and skin lesions
Deciding whether skin lesions should be ascribed to Leishmania can be challenging, especially in endemic regions, where prevalence of infection is high (often exceeding 50%). There are three possible scenarios: 1) dermatological problems due to the parasite in sick dogs affected by leishmaniosis; 2) dermatological problems, in infected dogs, due to another disease involving the skin; 3) dermatological problems due to a coexisting cutaneous disease, in sick dogs affected by leishmaniosis. In this latter case skin signs of CanL and those of concomitant diseases may overlap.

Cutaneous abnormalities associated with CanL are usually subdivided into common and miscellaneous disorders. Nonetheless, in the authors’ opinion, differentiating typical from atypical cutaneous problems helps decide the diagnostic protocol.

Typical dermatological problems are the most characteristic of CanL and usually the most frequent. They include, among others, scaling dermatitis with or without concomitant alopecia, ulcerative dermatitis over bony prominences and papular dermatitis (PapD).
Atypical dermatological problems resemble other skin diseases, and are less “specific” of CanL and less frequent. They include nasal planum dermatitis, ulcerative dermatitis in areas submitted to trauma, ulcerative dermatitis of distal extremities, multifocal alopecia and pustular dermatitis (PustD).

Dogs may show multiple concurrent problems of both types and the frequency of typical and atypical dermatological forms does not seem to differ in endemic and non-endemic regions. When dermatological problems are typical, diagnosis of CanL is not complex for clinicians working in endemic areas. On the other hand, because of the clinical, clinicopathological and histopathological appearances overlapping with many skin diseases, ascribing atypical cutaneous manifestations to CanL might be challenging even for skilled veterinarians living in endemic regions. For this reason, all cases of atypical CanL should be ideally confirmed by demonstration of the parasite in the affected skin.

Typical dermatological problems
In the group of typical dermatological problems, diagnostic challenges can be encountered in dogs with striking clinical features accompanied by mild to absent clinical-pathological abnormalities and low-positive to negative antileishmania antibody titres. This event seems to occur in certain cases of scaling dermatitis or, more frequently, in dogs with PapD.

Papular dermatitis (PapD). This clinical presentation, although not the most frequent, is quite distinctive and characterized by pinkish to yellowish persistent papules, sometimes umbilicated, located on poorly haired skin of short-coated dogs. Many dogs with PapD have no clinicopathological changes and a negative to low-positive antibody titre. If macrophages with/without neutrophils are observed but no amastigotes are visualized in cytological examination of papules, histopathological and eventually immunohistochemical examination of skin biopsies should be performed. PCR on fine needle aspirate from papules has also been recently reported as a useful diagnostic tool. In endemic regions, IHC should be preferred over PCR because in these areas the prevalence of infection is high and skin from infected dogs can be PCR positive even if healthy-appearing.

Atypical dermatological problems
Among atypical dermatological problems, diagnostic challenges are common as in the examples reported below.

Nasal planum dermatitis. Nasal planum dermatitis due to CanL can cause erosions, ulcerations, crusts and depigmentation and appears indistinguishable from discoid lupus erythematosus (DLE). If clinicopathological, serological and cytological results from a dog living in an endemic region and suffering from this type of nasal planum dermatitis do not lead to a sound diagnosis, the authors suggest a therapeutic trial with an oral antibiotic (chosen among those approved for empirical use in canine pyoderma) in order to reduce the inflammatory process secondary to the ulcerative dermatitis and, at the same time, to rule out mucocutaneous pyoderma (another differential diagnosis of CanL and DLE). In case of inadequate response to antibiotics, skin biopsies must be performed to confirm or rule out the presence of Leishmania in the affected skin. Based on the authors’ experience, a further
challenging diagnostic aspect is that nasal biopsies from dogs with either CanL or DLE seem to be characterized by a lympho-plasmacytic-histiocytic lichenoid dermatitis with/without interface damage. Therefore, if parasites cannot be visualized on H&E stained sections, their presence has to be excluded through immunohistochemical (preferred in endemic regions) or molecular techniques.

**Ulcerative dermatitis in areas submitted to trauma.** In infected individuals, the development of lesions at a site of traumatic interruption to the cutaneous integrity might represent another diagnostic challenge. It has been hypothesized that parasites are carried to lesional sites through infected macrophages recruited during the normal wound healing. In human beings, *Leishmania*-induced lesions precipitated by local trauma (e.g., wounds or tattooing) will develop within weeks to months after travelling to endemic countries and is considered a clinical pearl suggestive of cutaneous leishmaniosis.

Nodular or ulcerative leishmanial lesions have been reported in infected dogs at the site of surgical wounds or acral lick dermatitis. The challenging aspect in these cases is that, in the beginning, lesions are not due to the parasite; therefore, CanL might be erroneously underestimated and not be included in the list of differentials. Being infected, these dogs may have no other clinical signs, no or mild clinical-pathological abnormalities and negative or low-positive antibody titres. In addition, no amastigotes are observed in cytological examination of lesions. Their presence must be ruled out by means of histopathological and eventually immunohistochemical (preferred in endemic regions) or molecular examination of skin biopsies.

**Ulcerative dermatitis of distal extremities and multifocal alopecia.** Ulcerative dermatitis of distal extremities (e.g., margins and tips of ears, tip of the tail, nasal planum, footpads, claw bed) is suggestive of cutaneous vasculitis and has been sporadically documented in CanL, likely because lesions are uncommonly biopsied due to their location and because vascular damage is transient and focal. Multifocal cicatrical-like alopecia due to ischemic dermatopathy can be occasionally observed in dogs with CanL, also concurrent with signs of cutaneous vasculitis. Unlike vasculitis, ischemic dermatopathy is a relatively easy histopathological diagnosis, although the underlying vasculopathy may not be found in all the cases. Ulcerative dermatitis of distal extremities and multifocal alopecia with scarring appearance, likely represent the two ends of the same spectrum of cutaneous vessel damage.

Even when histopathological lesions of vasculitis or vasculopathy are visualized, demonstrating that CanL is the cause of vessel injury can be challenging. In fact, immunohistochemical and molecular examinations can give negative results because the damage is due to immune complexes. Because of the paucity of macrophage infiltrate and the fact that amastigotes are intramacrophagic, PCR should probably be preferred because IHC might be negative despite CanL being the cause of the lesions. Nevertheless, if the dog is infected, PCR might be positive even if the parasite does not play a causal role. A useful clue for the diagnosis is that if vasculitis is caused by CanL, antileishmania antibody titers are normally high.
In doubtful cases, response to antileishmanial drugs might help to confirm or exclude the causal role of parasites. However, to further complicate this issue, the outcome of antileishmania treatment might be partial in both vasculitis and ischemic dermatopathy, even if they are due to CanL. In fact, in dogs with vasculitis, in addition to specific antiparasitic therapy, short-term glucocorticoids might be needed to control the vascular inflammatory damage. In dogs with ischemic dermatopathy, hair regrowth might not occur, despite antileishmanial therapy, if follicular atrophy is irreversible.

**Pustular dermatitis (PustD).** PustD associated with CanL can represent a diagnostic and therapeutic challenge. It has been recently described as a variably pruritic multifocal to generalized PustD, histologically characterized by acantholytic subcorneal neutrophilic pustules. Favorable outcome has been reported in half of the patients treated with both antileishmanial and immunosuppressive drugs.

Based on the authors’ experience, a subgroup of dogs with CanL and PustD can be described further. In this subgroup, patients show generalized PustD affecting haired and, typically, sparsely-haired skin. Pustules are accompanied by erythematous papules, collarettes and scales-crusts, occasionally assuming annular to polycyclic configuration. Alopecia is not prominent and in the great majority of cases pruritus is intense. Broad pustules, spanning several hair follicles, containing neutrophils but no or occasional acantholytic keratinocytes, are observed at intra- and/or sub-corneal level. Superficial dermal oedema, vasodilation, neutrophilic exocytosis and intense superficial-mid, perivascular to interstitial infiltrate with predominant neutrophils are present in almost all cases. Dogs with this subtype of PustD can show systemic signs (fever) and are typically unresponsive to antibiotics, even when selected based on susceptibility testing results. Normally these patients do not respond to sole antileishmania therapy and glucocorticoids are required to get pruritus and dermatitis into remission. In these cases, the therapeutic protocol might be challenging due to potential contraindication of glucocorticoids in dogs with CanL. However, with a right balance between antileishmania and anti-inflammatory treatment, prognosis seems relatively favorable and, although infection persists, no relapses of PustD are observed at long-term follow-up.

Normally, dogs with this form of PustD show clinicopathological alterations compatible with CanL and positive antibody titres. However, it is not always possible to demonstrate the presence of parasites in diseased skin through immunohistochemical or molecular techniques. Therefore, establishing a causal relationship between CanL and PustD might be difficult and the concomitant presence of CanL and a “sterile” non-acantholytic immune-mediated superficial pustular dermatitis (e.g., superficial pustular drug reaction) cannot be ruled out. A preliminary case-control study has demonstrated a link, although not necessarily causal, between CanL and PustD.

Conclusions
In conclusion, CanL can be considered a “great impersonator” and diagnosis of both typical and atypical forms of CanL can be extremely challenging, even for experienced clinicians.
living in endemic countries and familiar with the disease. Atypical forms of CanL are often overlooked because of their similarity to other dermatological disorders.

The first step in the diagnostic protocol of CanL is staging the patient. Second, a causal relationship between the parasite and skin lesions has to be confirmed or excluded, sometimes in the absence of clinicopathological or serological abnormalities. This represents a critical step, both for diagnosis and therapy. If parasites are not detected via cytological and/or histopathological methods, their presence must be ruled out by means of immunohistochemical and/or molecular techniques. However, positive or negative results cannot confirm or rule out, respectively, a causal role of parasites in all dogs. In selected cases, evaluation of response to antileishmanial drugs is needed to attribute a pathogenic role to the parasite. Nevertheless, lack of response to antiparasitic treatment does not always rule out *Leishmania* involvement, as in the case of vasculitis, ischemic dermatopathy or PustD.

Selected References
PROBIOTICS, PREBIOTICS – HOW DO THEY WORK AND WHEN DO WE USE THEM?

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Introduction
The mammalian intestinal tract contains a complex, dynamic, and diverse population of non-pathogenic bacteria. Researchers have estimated that the average human contains 100 trillion microbes in the gut, which is 10 times more than the cells of the human body. The intestinal microbiota influences the health of the host by providing nutritional substrates, modulating the immune system, and providing a support in the defence against intestinal pathogens. The term microbiome refers to the total number of microorganisms and their genetic material and is contrasted from the term microbiota, which is the microbial population present in different ecosystems in the body.

There has been a plethora of research focusing on the mechanisms by which pathogenic bacteria influence intestinal function and induce disease; however, recent attention has focused on the indigenous non-pathogenic microbiota and the ways in which it may benefit the host. Initial colonization of the sterile newborn intestine occurs with maternal vaginal and fecal bacterial flora. The first colonizers have a high reduction potential and include species such as enterobacter, streptococcus, and staphylococcus. These bacteria metabolize oxygen, favouring the growth of anaerobic bacteria, including lactobacilli and bifidobacteria. Colonization with these bacteria is significantly delayed in caesarean deliveries, leading to delayed activation of the efferent limb of the mucosal immune response. Additional beneficial effects of developing a normal bacterial flora is seen in germ free mice that have small intestines that weigh less than their healthy counterparts. This effect occurs partly due to underdevelopment of lymphoid constituents, with a lack of plasma cells in the lamina propria and Peyer’s patches, and subsequent reduction in IgA production. Exposure to bacteria results in a reversal of this phenomenon within 28 days of exposure.

The intestinal microbiota has been associated with Crohn’s disease and ulcerative colitis, as well as irritable bowel syndrome in humans. In addition, the intestinal microbiota has also been implicated in the pathogenesis of various canine GI disorders, either associated with the presence of specific enteropathogens such as Salmonella, Clostridium perfringens, and viruses in acute episodes of diarrhea or a non-specific dysbiosis precipitating inflammatory bowel disease. Molecular phylogenetic studies have revealed a bacterial and/or fungal dysbiosis in the duodenum of dogs with IBD. A decrease in the proportion of Clostridiales and an increase in Proteobacteria is most commonly observed. Only a few studies have described the fecal microbiota of dogs with acute and chronic GI disorders. Dogs with acute diarrhea, particularly those with acute hemorrhagic diarrhea (AHD) have the most profound alterations in their microbiome characterized by decreases in Blautia, Ruminococcaceae including Faecalibacterium and Turicibacter spp, and significant increases in genus...
Sutterella and *Clostridium perfringens* compared to healthy dogs. Dogs with clinically active IBD had decreased *Faecalibacterium* spp. and Fusobacteria that increased during resolution of the IBD. The bacterial species that are commonly decreased during diarrhea are thought to be important short-chain fatty acid producers and could promote intestinal health. A deeper understanding of the gut microbiome will provide reference values for healthy populations and assist in diagnosing and treating diseased animals. In addition, manipulation of the intestinal microbiome via dietary intervention, administration of probiotics, prebiotics, or synbiotics, and fecal transplantation is currently being performed to maintain gut health in people and companion animals.

Manipulation of canine GI microbiota to improve health via dietary intervention did not begin until the early 1990’s. Dietary fiber, prebiotics and probiotics have been the major nutritional strategies studied to modulate the canine and feline GI microbiota. Unfortunately, most of the research studies published to date have evaluated the effects of dietary manipulation of the GI microbiome in clinically healthy dogs and cats, and many of these studies have used traditional plating techniques or qPCR to quantify a limited number of bacteria (e.g., *Lactobacillus*, *Bifidobacteria*, *C. perfringens*, and *E. coli*) to assess efficacy.

**Probiotics**

Probiotics refer to live microorganisms which when administered in adequate amounts confer a health benefit on the host. The term probiotic was derived from the Greek, meaning “for life.” The Food and Agricultural Organization of the United States (FAO) and the World Health Organization (WHO) have stated that there is adequate scientific evidence to indicate that there is potential for probiotic foods to provide health benefits and that specific strains are safe for human use. There has been a literal explosion of interest and research on the subject in recent years. Despite this activity, much still remains to be done to determine the specific indications and applications of probiotics in dogs and cats. There has been tremendous interest among veterinary pet food companies and manufacturers of animal health and wellness products to market probiotic formulations that are safe, pure, stable, and confer a beneficial effect in dogs and cats. These products are generally preferred to the multitude of over-the-counter probiotics marketed for veterinary use, given the concerns pertaining to quality control of the over-the-counter products. A number of criteria are essential for efficacy and safety of probiotics. These include resistance to gastric acid and bile, ability to colonize the gastrointestinal tract, efficacy against pathogenic microorganisms, and modulation of the immune system. Several potential mechanisms have been proposed for how probiotics reduce the severity or duration of diarrhea: competition with pathogenic bacteria or viruses for nutrients, competition for receptor sites, modification of the metabolic activity of the intestinal microflora, and the direct antagonism through the action of antimicrobial metabolites.

**Evidence for the Benefits of Probiotics in People**

There is currently level 1 evidence (i.e., data from either high-quality, randomized controlled trials with statistically significant results and few design limitations or from systematic reviews of trials) for effectiveness of probiotics in treating lactose intolerance/maldigestion, treating acute infectious or nosocomial diarrhea in children, preventing or treating antibiotic-associated diarrhea, preventing and maintaining remission of pouchitis in adults, and
maintaining remission of ulcerative colitis in adults. In addition, there is level 2 evidence (evidence obtained from randomized trials that have limitations in methodology or results that have wide confidence intervals) for using probiotics to treat traveler's diarrhea, prevent sepsis secondary to severe acute pancreatitis, prevent infections in postoperative patients. A recent meta-analysis of randomized clinical trials evaluating the efficacy of synbiotics (probiotic plus a prebiotic) for the treatment of atopic dermatitis in children found that there was compelling evidence for the effective use of mixed strains of bacteria in children aged 1 year or older. From the 6 treatment studies included for random-effects meta-analysis, the overall pooled change in SCORAD (Severity Scoring of Atopic Dermatitis) index in those treated with synbiotics at 8 weeks of treatment was -6.56 (95% CI, -11.43 to -1.68; P = .008). Further studies are needed to evaluate the effectiveness of synbiotics for primary prevention of AD.

**Evidence for the Benefits of Probiotics in Dogs**

To date, only a relatively small number of studies have been published evaluating the effects of probiotics in dogs, and many of these have focused on the intestinal microbiota in apparently healthy dogs. Probiotic strains of human or canine origin (Lactobacilli, Bifidobacter, and Enterococcus) were used in healthy adult dogs or dogs with food-responsive diarrhea to assess their effects on intestinal microbial populations, their ability to reduce specific pathogens in feces, and effectiveness as immunomodulators. In many of these studies, probiotics added to the food in healthy dogs had an equivocal effect on fecal microflora and pathogens. However, it is important to note that most of these studies were not randomized, controlled trials, and the strains of probiotic varied from study to study, making interpretation of findings more challenging. In addition, many studies focused on fecal isolation and quantitative cultures of putative pathogenic bacteria such as *C. perfringens*, rather than on the evaluation of more meaningful end points such as phylogenetic characterization of the microbiota, mucosal immunopathology, and alterations in intestinal integrity. Only two studies addressing the role of probiotics in management of dietary sensitivity and food-responsive diarrhea have been published to date, with overall positive results. Only one of those studies was a randomized, placebo-controlled clinical trial, and the results of that study, although clinically positive (all of the dogs in the study improved when they were placed on the elimination diet) showed no specific changes in the inflammatory cytokine patterns or a specific benefit of the probiotic. The immunomodulatory effects of *Enterococcus faecium* SF68 have been studied in dogs, and the probiotic was associated with increased fecal IgA concentrations and increased vaccine-specific circulating IgG and IgA concentrations. Although increased immune globulins may suggest enhanced immune response, the clinical relevance of this finding is not known. A recent study completed at Colorado State University confirmed that SF68 is resistant to metronidazole and so the two compounds can be administered together. In addition, diarrheic dogs treated with metronidazole and SF68 in combination had a reduced treatment time of 2.8 days compared to 4.4 days when metronidazole was administered alone.

A recent double-blind, placebo controlled trial of a probiotic strain of *Lactobacillus sakei* Probio-65 administered for 2 months to research dogs with atopic dermatitis significantly reduced the CADESI and PVAS scores of the dogs compared to a placebo group.
Additional studies are warranted in dogs to further assess the immunomodulatory effects of probiotics and to evaluate their safety. The latter issue is particularly important given the recent finding of increased intestinal adhesion of *Campylobacter jejuni* in an *in vitro* model of canine intestinal mucus following incubation with *Enterococcus faecium*. It should be noted that this *E. faecium* strain is different from the *E. faecium* SF68 strain available commercially; moreover, to date there has been no clinical or anecdotal evidence of *Campylobacter*-associated diarrhea in dogs associated with *E. faecium* administration. Short-term treatment (6 weeks) with *E. faecium* SF68 to 20 dogs with chronic naturally acquired subclinical giardiasis failed to affect giardial cyst shedding or fecal giardial antigen and did not alter innate or adaptive immune responses at multiple time points. These results are in contrast to those shown following the oral feeding of *E. faecium* strain SF68 to mice experimentally infected with *Giardia intestinalis* trophozoites. Oral feeding of *E. faecium* strain SF68 starting 7 days before inoculation with *Giardia* trophozoites significantly increased the production of specific anti-*Giardia* intestinal IgA and blood IgG. This humoral response was mirrored at the cellular level by an increased percentage of CD4+ T-cells in the Peyer's patches and in the spleens of SF68-fed mice. The improvement of specific immune responses in probiotic-fed mice was associated with a diminution in the number of active trophozoites in the small intestine as well as decreased shedding of fecal *Giardia* antigens (GSA65 protein). The latter findings underscore the importance of carefully evaluating the animal model, the timing of probiotic administration (prior to infection or following infection), and the specific end-points assessed.

### Evidence for the Benefits of Probiotics in Cats

Unfortunately, there is little published information pertaining to probiotic use in cats, and only one clinical study has reported a beneficial effect of probiotic therapy for any feline disease to date. In that study, administration of *Enterococcus faecium* SF68 to 217 cats housed in an animal shelter was associated with a significantly lower percentage of cats with diarrhea ≥ 2 days (7.4%) compared with a placebo group (20.7%). One study evaluating the effect of dietary supplementation with the probiotic strain of *Lactobacillus acidophilus* DSM 13241 (2 × 10^8 CFU/d for 4.5 weeks) administered to 15 healthy adult cats demonstrated that recovery of the probiotic from the feces of the cats was associated with a significant reduction in *Clostridium* spp. and *Enterococcus faecalis*. However, the immunomodulatory effects were reported based on decreased lymphocyte and increased eosinophil populations and increased activities of peripheral blood phagocytes. The relevance of these findings is unclear, because this study was not a randomized trial and the changes reported in the populations of peripheral blood cells cannot be extrapolated into evidence of systemic health benefits. Evaluation of the effect of supplementation with *Enterococcus faecium* strain SF68 on immune function responses following administration of a multivalent vaccine was evaluated in specific pathogen-free kittens. This prospective, randomized, placebo-controlled study resulted in the recovery of *E. faecium* SF68 from the feces of seven of nine cats treated with the probiotic, and a nonsignificant increase in feline herpesvirus 1–specific serum IgG levels. Concentrations of total IgG and IgA in serum were similar in the probiotic and placebo groups, and the percentage of CD4+ lymphocytes was increased significantly only in kittens at 27 weeks and not at any other time points. Probiotics also have been
evaluated in juvenile captive cheetahs, a population with a relatively high incidence of bacteria-associated enteritis. Administration of a species-specific probiotic containing *Lactobacillus* Group 2 and *Enterococcus faecium* to 27 juvenile cheetahs was associated with a significantly increased body weight in the treatment group, with no increase in the control group. In addition, administration of the probiotic was associated with improved fecal quality in the probiotic group.

**Prebiotics**

A prebiotic is defined as a “nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth of and/or activates the metabolism of one or a limited number of health promoting bacteria in the intestinal tract.” The most common prebiotics studied are fructans, although other prebiotics such as mannans, lactosucrose, and lactulose are also being evaluated.

**Evidence for the Benefits of Prebiotics in Dogs and Cats**

Unfortunately, there is a paucity of information evaluating the clinical benefits of prebiotics in dogs and cats. Most of the outcomes published in the literature are limited to nutrient digestibility, microbial concentrations in feces, and fecal protein catabolites that may not necessarily denote a health benefit to the patient. The effects of short chain fructooligosaccharides (scFOS) were evaluated in a group of German Shepherd dogs suspected to have IgA deficiency. Although the scFOS supplemented dogs had decreased aerobic and anaerobic bacteria in intestinal biopsies, the findings of the study were clouded in light of the fact that anaerobic bacterial counts did not decrease in the intestinal fluid samples of the dogs supplemented with scFOS. The effects of various oligosaccharides have been tested in adult ileal cannulated dogs to evaluate the effects on ileal and total tract nutrient digestibilities, microbial populations, ileal pH, ammonia, blood glucose, fecal consistency, and SCFA concentrations. Oligosaccharides (oligofructose, mannanoligossaccharides, and xyloooligosaccharides) were each given at 0.5% of the diet in a Latin square design. The only significant finding was a decrease in fecal *Clostridium perfringens* populations in dogs fed MOS. The effects of 3% inulin supplementation of elimination and hydrolyzed protein diets to healthy dogs was associated with a slight increase in fecal moisture content (not clinically relevant), decreased apparent nutrient digestibility coefficients of crude protein in dogs on the elimination diet, and no effect on fecal IgA concentrations. Adult beagles fed diets containing cellulose or beet pulp plus oligofructose for 6 weeks were found to have similar fecal concentrations of total anaerobes and aerobes; however, the dogs fed oligofructose had fewer Enterobacteriaceae and clostridia and greater numbers of lactobacilli. In addition, dogs fed oligofructose had longer and heavier small intestines (35% heavier), and 37% more mucosal mass with consequent greater absorptive surface area. Administration of 1% scFOS or 1% inulin to weanling puppies (12-weeks-old) during a pathogen (*Salmonella Typhimurium*) challenge was associated with a lower severity of enterocyte sloughing in puppies consuming the fructans versus the control diet. In addition, puppies fed inulin also had higher fecal acetate, total SCFA concentrations and lactobacilli, indicating that prebiotics appear to attenuate some of the adverse effects of *Salmonella* challenge, and may provide protection against infection in weanling puppies. Cats fed diets containing 0.75% oligofructose had significantly increased fecal concentrations of lactobacilli and decreased
concentrations of *C. perfringens* and *E. coli* compared with controls.\textsuperscript{32} A study evaluating the effects of short-chain fructooligosaccharides (0.5\%) and galactooligosaccharides (0.5\%) in healthy cats showed no effect on fecal protein catabolites, including ammonia, 4-methylphenol, indole, and biogenic amines, underscoring the fact that concentrations of oligosaccharides > 0.5\% should be used to elicit a positive response.\textsuperscript{33} The first nutritional intervention study in dogs that used pyrosequencing to evaluate the effects of beet pulp fiber on fecal microbial composition was performed in 2010.\textsuperscript{34} Dog fed a control diet were compared to dogs fed a diet containing 7.5\% beet pulp in a crossover design with 14 day periods. *Eubacterium balii* and *Faecalibacterium prausnitzii*, both of which are butyrate producers, were overrepresented in the dogs fed the beet-pulp containing diet, suggesting a possible anti-inflammatory effect of the beet-pulp. In contrast, Fusobacteria was under-represented in dogs fed the beet-pulp-containing diet. Recent studies have attempted to characterize the fecal microbiota of diarrheic dogs, as well as dogs with IBD. Reduced bacterial diversity as well as significantly higher proportion of Enterobacteriaceae were observed in duodenal brush borders from dogs with IBD compared to healthy controls.\textsuperscript{7} Suchodolski et al. confirmed a bacterial dysbiosis in fecal samples of dogs with chronic diarrhea (IBD) and acute hemorrhagic diarrhea, and observed changes in the microbiome between acute and chronic disease states. The bacterial groups that were commonly decreased are important producers of short-chain-fatty acids and may play an important role in canine intestinal health.\textsuperscript{8}

**Fecal Microbiota Transplantation (FMT)**

Fecal microbiota transplant or infusion of a fecal suspension from a healthy individual into the gastrointestinal tract of another person to cure a specific disease, is best known as a treatment for recurrent *Clostridium difficile* infection (RCDI) in people.\textsuperscript{35} and experience with FMT for ulcerative colitis and Crohn’s disease is somewhat limited. Re-establishment of the wide diversity of intestinal microbiota via infusion of donor feces into the colon is the proposed mechanism in patients with RCDI and IBD. FMT has been performed in dogs with a variety of chronic enteropathies (Scott Weese, personal communication), and the author (SLM) is currently completing a clinical trial evaluating the efficacy of FMT in Macaques with chronic colitis. There are a variety of application methods to inoculate the donor feces into the patient, and most studies have relied upon colonoscopy over retention enemas or nasogastric tubes to facilitate inoculation of the feces in the ileum and colon.

**Conclusions and Future Directions**

The potential benefits and specific indications for the administration of pro- and prebiotics to dogs and cats have yet to be fully defined, although our knowledge and understanding of the nature and diversity of the feline and canine intestinal microbiome during health and disease has expanded rapidly following the advent of high-throughput DNA-sequencing platforms. Defining a role for pro- and prebiotics as well as FMT in dogs and cats will require completion of prospective, randomized, placebo-controlled studies that rely on clinically relevant end points related to a particular physiological or pathological condition. Further studies are warranted to determine the need for probiotics to be live microorganisms following the provocative studies of Rachmilewitz et al., who documented that the beneficial effects of probiotics are mediated by their DNA, circumventing the need for live, viable
bacteria. Pro- and prebiotics do appear to have a potential role in the prevention and treatment of various gastrointestinal illnesses, but it is likely that benefits achieved are specific to the bacterial species used and to the underlying disease context.

References
What’s the future of therapeutics in veterinary dermatology? Things that in the past seemed esoteric, or like distant possibilities, are becoming commonplace as research rapidly advances. Many of these findings will lead to new diagnostic and treatment products that will be invaluable in everyday practice. It is therefore important that we all learn about and understand the developments that are taking place, and how they will impact our practices in the future.

What Are Biologic Therapies?
Biologic therapies (also called “immunotherapeutics”) are therapies that use a biological compound, rather than a chemical. They are typically protein or peptide molecules that are made in laboratory culture rather than by chemical synthesis. Because they are proteins and not metabolized like a drug, they often have a long-lasting effect and are very targeted.

Active immunotherapy is administration of a substance that causes the host to mount some kind of immunologic response to the substance. Familiar examples include vaccinations, or allergen-specific immunotherapy. Less familiar, but newer examples include administration of recombinant interferons to treat various disease states. Passive immunotherapy is administration of a molecule that has a direct effect on its own, without the immune system having to respond. Examples include administration of intravenous immune globulin for people with immunodeficiency diseases, where they are unable to make antibodies on their own. More recently, administration of immune serum has made the news, where serum from recovered Ebola virus patients was used to treat sick victims. An increasingly common example is the use of monoclonal antibodies or “fusion proteins” therapeutically.

A Primer on Monoclonal Antibodies
Monoclonal antibodies (mAbs) are immunoglobulins that have been manufactured for a specific purpose. In days past, antibodies were made by injecting animals with a foreign substance, then harvesting their serum – which would contain antibodies against the substance. These antibodies bind to many different regions of the substance and are therefore called “polyclonal.” If instead, we immunize a mouse, harvest antibody-producing spleen cells from the mouse, and fuse them with an immortalized lymphocyte cell line, we end up with cells that produce antibodies of only one specificity, so-called monoclonal antibodies. A huge advantage is that these cells can then be cultured in large vessels to produce many grams (or even metric tons!) of pure antibody of the same specificity.

One of the problems with using antibodies therapeutically is that the monoclonal antibodies are of mouse origin. Injecting mouse antibodies into a person or dog repeatedly will surely result in an immune response against the mouse protein, rendering it ineffective and perhaps even producing an allergic reaction. To get around this problem, the mouse antibody has to
be “speciated” – converted into an antibody that is very much like a human (or dog) antibody so that it will not be recognized as foreign. There are various ways of engineering the mouse protein to make them “humanized,” “caninized,” “felinized,” etc.

Therapeutic Use of Monoclonal Antibodies

The naming convention of therapeutic mAbs deserves mention – it’s not as confusing as it appears. All therapeutic mAbs are named with a prefix, a target, a source, and a suffix. The suffix is always “mab.” The prefix is chosen by the manufacturer. The target is indicated by e.g. [li or lim] for a target in the immune system; [k or ki] for a target that is an interleukin; and [t or tu] for a tumor target. The source is indicated by e.g. [xi] if a chimeric antibody; [zu] for a humanized antibody; or [u] as a fully human antibody. Thus, the most common therapeutic antibody sold worldwide is Adalimumab (Humira®, AbbVie). This is a monoclonal (mab) that is fully human (u) directed against a target in the immune system (lim) with the prefix Ada.

Therapeutic use of mAbs mimics natural antibodies produced by the host. However, instead of the host’s immune system making the antibody, it is manufactured, and injected into the patient. There are hundreds of mAbs in various stages of development and clinical trial for human diseases. These mAbs may be directed against a microorganism, a tumor cell, a cell of the immune system, or a cytokine or its receptor. Familiar examples on the market currently include adalimumab (anti-TNF-alpha, for psoriasis, rheumatoid arthritis, and Crohn’s disease), omalizumab (anti-IgE, for human allergic diseases), or rituximab (anti-CD20, a lymphocyte marker used to treat lymphoma), and many others. An interesting human mAb in clinical trial is dupilumab, which blocks the receptor for IL4/IL13. It has shown great promise in treating refractory human atopic dermatitis. Unfortunately, all of these mAbs would be ineffective in dogs or cats, as they are engineered specifically for human beings.

What about the promise in veterinary medicine? Can these principles, so valuable for human therapies, be translated to the animal world? The answer is a resounding YES, and we are just beginning to see the appearance of therapeutic mAbs in our own clinics. The mAbs furthest along in development include Blontress® or Tactress® (Aratana) for treating lymphoma, anti-nerve growth factor (NexVet) for treating chronic pain, and anti-IL-31(Zoetis) for treating canine atopic dermatitis.

IL-31 is a key cytokine in pruritic skin disease. Its actions occur through receptors that utilize the JAK/STAT signalling system, and disruptions of this pathway by JAK inhibitors such as oclacitinib can result in remarkable relief from pruritus in dogs. A different approach to accomplish a similar effect would be to inactivate IL-31 by using a monoclonal antibody directed against it. Humanized anti-IL-31 mAb is currently in Phase I trials for human atopic dermatitis. Zoetis has recently received a conditional license for a caninized anti-IL-31 mAb for canine atopic dermatitis. In preclinical trials, field trials, and early clinical experience, this product appears to be safe and very effective.

What could go wrong with biological therapies? First, because these products are peptides or proteins, they are not metabolized by the liver or kidneys, as with a drug, and are not
expected to have specific organ toxicity. They are merely degraded into their constituent amino acid, which are recycled for other uses in the body. There is also the possibility that these molecules could become immunogenic. This appears to happen in a very small number of individuals, based on human experience, and the same may prove true in veterinary medicine. If antibodies are generated which react against the therapeutic substance, at the very least it will be inactivated. At worst, continued administration could result in allergic reactions. Finally, the “targets” against which mAbs are directed are usually normal constituents of the immune system, such as cytokines, and surely have important functions. So we must ask if there will be any adverse effects by eliminating the action of these normal molecules over a prolonged period of time.

Biological therapies are cutting-edge, unique, exciting, and potentially immensely useful treatments in medicine, with many potential targets, and constantly advancing technology. They are not perfect treatments, however, and still are only part of a multimodal treatment approach for most diseases. It is important for practitioners to be aware of and understand these therapies, as they are more and more likely to become routine in the years ahead.

Selected References
**FATTY ACIDS IN VETERINARY DERMATOLOGY AND BEYOND: MECHANISM OF ACTION, CLINICAL INDICATIONS AND QUALITY**

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**Fatty Acid Basics**
A fatty acid is a carboxylic acid with a long aliphatic tail. Most naturally-occurring long-chain fatty acids have 13-21 carbons. Those of interest for this discussion are polyunsaturated with two or more double bonds. Fatty acids are usually derived from dietary triglycerides or phospholipids. When they are not attached to other molecules such as glycerol, they are known as free fatty acids. Fatty acids are important sources of energy, but for this discussion their most important functions are as structural components of cell membranes and as precursors of important inflammatory mediators.

**Omega-6 fatty acids.** Omega-6 fatty acids are polyunsaturated with the first carbon-carbon double bond at the sixth carbon (n-6) from the methyl end of the molecule. They are essential for all stages of life in dogs and cats because they cannot be synthesized in the body and their deficiency is associated with well-defined clinical abnormalities or suboptimal physiologic processes. Linoleic acid (LA) is the main dietary source. Gamma-linolenic acid (GLA), dihomogamma-linolenic acid (DGLA) and arachidonic acid (AA) are important functional metabolites and may also be found in the diet.

Omega-6 fatty acids are found in phospholipids in all cell membranes. Additionally, they are incorporated into lamellar bodies (lipid organelles in the viable epidermal cells) and then released into the intercellular spaces in the stratum corneum helping to form the cutaneous barrier. As such, omega-6 fatty acids along with ceramides and cholesterol are important in cutaneous protection of the body providing the first defense against multiple environmental pathogens and allergens.

Western diets and most commercial pet foods contain excessive amounts of omega-6 fatty acids. Too much dietary omega-6 may result in more AA synthesis and deposition in cell membrane phospholipids. When AA is released from cell membrane phospholipids by phospholipases A₂ (PLA₂), downstream modification by cyclooxygenases and lipoxygenases leads to production of more pro-inflammatory eicosanoids. This may be a problem for patients with acute and chronic inflammatory conditions.

**Omega-3 fatty acids.** Omega-3 fatty acids are polyunsaturated with the first carbon-carbon double bond at the third carbon (n-3) from the methyl end of the molecule. Although they are not required to be in dog and cat foods in the United States, they cannot be synthesized in the body and evidence suggests their essentiality for optimal reproductive and growth phases of life (central nervous system and retinal development to be discussed later) and for support of a normal inflammatory response. Alpha-linolenic acid (ALA) is the main plant-derived dietary source. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be found in the diet (marine lipids, marine algae) and are also metabolites of ALA. ALA from vegetable sources
such as flax, flaxseed oil or other plant oils does not provide the same tissue levels or clinical
benefits at comparable doses as directly providing EPA and DHA, since conversion of ALA to
the longer chain metabolites in mammalian species is inefficient, less than 10% in dogs and
virtually nonexistent in cats.

**Maintenance of a normal inflammatory response.** Omega-3 fatty acids compete with omega-6 fatty acids for the same metabolic enzymes, resulting in less production of and displacement of omega-6 fatty acids in phospholipids; most important in many organ systems is EPA in place of AA. This process is sometimes referred to as Competitive Inhibition and Displacement. Resultant EPA mediators (eicosanoids) are neutral, less inflammatory or anti-inflammatory. Counteraction: there is also evidence that eicosanoids produced from EPA can directly counteract those produced by AA. Additionally, downstream endogenous lipid mediators (lipoxins, resolvins, protectins, maresins, etc.) from EPA and DHA have analgesic properties and help signal the termination of acute inflammatory responses in cells and tissues.

**Clinical Indications**

*Cutaneous health.* As discussed in the previous section, linoleic acid (LA, omega-6) is essential for the normal health of the skin and hair coat because of its role as an important structural component of cell membrane phospholipids and the stratum corneum intercellular lipid barrier. For this reason, dietary supplementation with oils high in LA (sunflower, safflower, soy, corn, etc.) has been recommended for dry scaly skin conditions sometimes referred to as seborrhea sicca. This condition may be idiopathic or associated with poor diets, excessively dry environments, underlying endocrinopathies such as hypothyroidism, etc.

This approach may be warranted for dry skin without inflammation. However, the concern is that supplementation of high levels of omega-6s in patients on diets already containing excessive amounts may help the dry scaly skin look better but may further contribute to a pro-inflammatory response in the skin and other organ systems. A commercial omega-6/omega-3 combination product or diet may be a better alternative to omega-6 oil alone since the omega-3 component may help support a normal inflammatory response and/or have a sparing effect on LA. Alternatively, dermatologists may recommend the use of topical shampoo, rinse and spot-on formulations containing omega-6 fatty acids and other lipid components to directly support the skin and hair coat thus avoiding a systemic pro-inflammatory effect.

*Support of skin disorders with an abnormal inflammatory response.* Several clinical studies have documented efficacy of omega-32,3,4 or omega-6/omega-35,6,7 combinations as adjunctive therapy for pruritic and inflammatory dermatoses, including the ability to use a lower dose of prednisolone after 2 months6 and a lower dose of cyclosporine after 12 weeks7 of supplementation in dogs with atopic dermatitis.

A recommended dose of omega-3 fatty acids in a fish oil supplement is 180 mg of EPA and 120 mg of DHA per 10 lbs (4.55 kg) of body weight (BW) per day. Efficacy was documented in 16 dogs with idiopathic pruritus or pruritus associated with atopic dermatitis and/or flea allergy in a double-blinded, corn oil-controlled crossover study (6 weeks of treatment with each test article with a 3 week washout).2 Dogs receiving the fish oil showed significant improvement in pruritus, self-trauma and coat character over time. When compared to the corn oil control over
time, fish oil supplementation significantly improved pruritus, alopecia and coat character. Fifty-six percent (56%) of the dogs on fish oil had ≥50% improvement in clinical scores compared to only 6% of dogs on corn oil. The dose used in the study corresponds to 66 mg of combined EPA and DHA per kg of BW.

Another double-blinded, placebo-controlled, randomized trial was conducted in 29 dogs with atopic dermatitis over a 10 week period using a commercial fish oil product, flax oil or mineral oil placebo. The fish oil was administered at 180 mg of EPA and 120 mg of DHA per 5 kg of body weight per day (60 mg of combined EPA and DHA per kg of BW). Both the fish and flax oil groups had significant improvement in post-treatment clinician and owner scores but not dogs treated with mineral oil. It took 2.3 times as much omega-3 fatty acids in the flax oil form as in the fish oil form for similar clinical improvement.

Sixty-eight (68) dogs with atopic dermatitis were administered a mineral oil placebo (35) or 74.6 mg of combined EPA and DHA per kg of BW (33) for 12 weeks in a double-blinded, placebo-controlled, randomized clinical trial. At both 6 and 12 weeks the treated dogs had significant reductions in their clinical scores (Canine Atopic Dermatitis Lesion Index) and higher overall improvement in owner/investigator visual analog scale scores for pruritus compared to placebo.

Based on clinical experience and the studies referenced in this section, allow at least 4-6 weeks for an initial effect and 8-12 weeks for a full effect. This recommendation applies to use of omega-3 fatty acids for support of a normal inflammatory response in any organ system, not only the skin.

**Nervous system development in puppies and kittens.** The omega-3 fatty acid docosahexaenoic acid (DHA) is needed for optimal neurologic development (especially retinal and auditory) *in utero* and during growth and development in children. The same has been documented for neurocognitive development in puppies and kittens.

Fish oil as a source of DHA was fed in diets to 48 beagle puppies from 8 to 52 weeks of age. Low, moderate and high DHA-containing foods were utilized. The high-DHA group (Puppy Growth Formula, Hill’s) had significantly better results for reversal task learning, visual contrast discrimination and early psychomotor performance in side-to-side navigation through an obstacle-containing maze than did the moderate-DHA and low-DHA groups. The high-DHA group had significantly higher anti-rabies antibody titers 1 and 2 weeks after vaccination than did other groups. Peak b-wave amplitudes during electroretinography (ERG) were positively correlated with serum DHA concentrations at all evaluated time points.

DHA is metabolized from ALA in the diet. However, it has been shown that ERG responses and rod sensitivity are improved at 12 weeks of age in puppies fed diets (gestation, lactation and weaning) with DHA from fish oil but not with comparable levels of dietary ALA. An amount of ALA ten (10) times greater than the amount of DHA was needed for the beneficial effects to be seen.

Based on these and other studies, it is recommended that preformed DHA be provided in the diet during gestation, lactation and post-weaning for optimal neurological development in puppies.
and kittens. The current dosage recommendation is to follow the 2006 NRC Recommendations for Dogs and Cats.\textsuperscript{11} Pregnant bitch and growing puppy after weaning: 130 mg EPA + DHA per 1,000 kcal of metabolizable energy; Pregnant queen and growing kitten after weaning: 25 mg EPA + DHA per 1,000 kcal of metabolizable energy.

Support of a normal inflammatory response for joint health. A review of the scientific literature by Bauer\textsuperscript{1} concluded that COX-2 and 5-LOX may be appropriate targets for the management of symptoms associated with naturally occurring osteoarthritis in dogs and that the omega-3 long-chain polyunsaturated fatty acids may modify the activities of these enzymes.

As described earlier, omega-3 fatty acids antagonistically compete with omega-6 fatty acids which may help balance the production of inflammatory mediators. Additionally, omega-3 fatty acid (but not other fatty acids) incorporation into bovine cartilage chondrocyte membranes resulted in a dose-dependent reduction in the expression and activity of proteoglycan degrading enzymes (aggrecanases) and the expression of inflammation-inducible cytokines IL-1\(\alpha\), TNF-\(\alpha\) and COX-2, but not the constitutively expressed COX-1.\textsuperscript{12} Thus, omega-3 supplementation may specifically affect molecular mechanisms that regulate the expression of catabolic factors involved in articular cartilage degradation that cause and propagate arthritic disease.

A therapeutic food with high levels of omega-3 fatty acids and a small amount of glucosamine was fed for 3 months to 22 client-owned dogs with osteoarthritis along with 16 dogs on a control food in a randomized, double-blinded trial.\textsuperscript{13} Veterinary assessment revealed significant improvement in lameness, clinical weight-bearing scores and force-plate weight bearing at the end of the 3 months in the treatment but not the control group. Additionally, dietary supplementation with omega-3 fatty acids has been used adjunctively with carprofen in dogs with osteoarthritis.\textsuperscript{14} Results of this study suggest that dietary fish oil omega-3 fatty acid supplementation may allow a reduction in carprofen dosage.

Based on levels used in the above feeding trials and other published studies, supplementation in the range of 85-100 mg of combined EPA and DHA per kg of BW is suggested by the author. Bauer suggests that even higher dosages may be used depending on severity and chronicity of the disorder up to the NRC safe upper limit in dogs of 370 mg of combined EPA and DHA per kg\textsuperscript{0.75} (metabolic BW basis).\textsuperscript{1} Interestingly, veterinary diets marketed for joint health when fed according to label recommendations to a 60 lb (27.3 kg) dog contain as low as 20 to as high as 102 mg of combined EPA and DHA per kg of BW. Therapeutic diets with fish oil may also contain ALA which may further contribute to a beneficial effect. However, some diets may contain ALA (generally flax) as the only source of omega-3 fatty acids. This is a concern because of the inefficient conversion to EPA and DHA mentioned previously.

Cardiovascular support. Heart failure and associated cachexia are known to be associated with an abnormal inflammatory response. An excellent review article was published in 2010 highlighting the beneficial effects of omega-3 fatty acids to support a more normal inflammatory response in cardiovascular disease in dogs.\textsuperscript{15}

A randomized, double-blinded, placebo-controlled study was conducted on 28 dogs with stable chronic heart failure secondary to idiopathic dilated cardiomyopathy.\textsuperscript{16} Baseline plasma AA,
EPA and DHA concentrations were found to be significantly lower in dogs with heart failure than in controls. Fish oil supplementation (27 mg of EPA and 18 mg of DHA per kg per day) for 8 weeks normalized these deficiencies, significantly decreased IL-1 concentrations, decreased PGE₂ production, improved food intake, reduced muscle loss and improved cachexia compared to the placebo group. Reductions in circulating IL-1 concentrations over the study period correlated with increased survival times. These data suggest that anti-cytokine strategies with omega-3 fatty acids to help support a more normal inflammatory response may benefit patients with heart failure.

Dogs have occasionally been used in experimentally induced conditions to study the effects of omega-3 fatty acids on ventricular and atrial arrhythmias. In a canine model of atrial tachypacing, orally administered long-chain omega-3 fatty acids prevented congestive heart failure-induced atrial structural remodeling and atrial fibrillation promotion. In another canine cardiac pacing model of atrial cardiomyopathy, oral omega-3 supplementation reduced atrial fibrillation inducibility and maintenance, reduced conduction anisotropy in the left atrium and prevented pacing-induced increase in collagen turnover and collagen deposition in atrial appendages.

In a retrospective study of 108 dogs with heart failure secondary to dilated cardiomyopathy or chronic valvular disease, there was a significantly (P = 0.009) longer survival time for dogs receiving omega-3 fatty acid supplementation in comparison to those that did not.

In her review article, Dr. Freeman concluded “…that there is adequate evidence to warrant the use of omega-3 fatty acids in dogs, and likely cats, with heart failure or certain arrhythmias for secondary prevention. In addition, omega-3 fatty acids may have benefits in earlier stages of cardiac disease (e.g. DCM, CVD, HCM) due to their numerous positive effects on the cardiovascular system but this requires further research.”

Although more research is needed to establish an optimal dose of omega-3 fatty acids for cardiovascular support, the current recommendation based on the published evidence is 40 mg/kg EPA and 25 mg/kg DHA per day for both dogs and cats. Furthermore, there is no optimal omega-6:omega-3 ratio as is often claimed. It is the total omega-3 dose that determines plasma omega-3 fatty acids, independent of the ratio.

**Potential Adverse Effects**

There are several potential adverse effects of high levels of dietary supplementation of long-chain omega-3 fatty acids as suggested in a review article. Most of these would expect to be dose- and duration-dependent: GI upset, diarrhea, pancreatitis, altered platelet function, delayed wound healing, lipid peroxidation, weight gain, altered immune function, effects on glycemic control and insulin sensitivity, and nutrient-drug interactions.

Clinicians prescribing omega-3 fatty acids should be aware of these in light of a patient’s medical history. However, clinically these are either extremely rare or have never actually been documented. This is likely explained by the relatively low doses recommended in relation to established safe levels. The National Research Council publication on Nutrient Requirements of Dogs and Cats indicates a safe upper limit of the combined amounts of EPA + DHA as 2,800
mg/1,000 kcal of diet, equivalent to 370 mg/kg$^{0.75}$ of combined EPA and DHA for dogs.$^{11}$ Presently, not enough published data are available to set a safe upper limit for cats.

**Product Quality and Fish Oil Options**

**Sources of fish oil.** Wild salmon have historically been an important type of fish used for omega-3 fatty acids because of their high fat content. However, as they have been over-fished, quantities have declined. Farm-raised salmon and other fish species have become popular to address the diminishing wild population. The quality of fish-farming operations is variable. Regulation of aquaculture operations varies widely by species, farming system and country.$^{22}$ Therefore, a more satisfactory option for source of fish oil may be the use of wild, smaller non-predatory and more easily renewable high fat content species such as anchovies and sardines.

Fish oils should be fully tested for heavy metals (e.g. mercury and lead), ocean pollutants (e.g. PCBs and dioxins), microbial contamination and other contaminants. Some of these standards have been set by the Council for Responsible Nutrition (CRN), World Health Organization (WHO) and International Fish Oil Standards (IFOS). In the US, the FDA has set tolerable levels for many contaminants found in fish and fish oils, but only 1-2% of shipments of fish products entering the US are inspected and tested.

**Chemical forms of fish oil.** Triglycerides is the most common form of fish oil with relatively low concentrations of EPA and DHA and good absorption from the GI tract. Generally, about 25-30% of the total fish oil weight consists of EPA/DHA. For example, a 1,000 mg triglyceride fish oil softgel will contain approximately 250-300 mg of EPA/DHA. Most OTC and veterinary products are triglycerides. Re-esterified triglycerides are different than natural triglycerides. Processing is generally accomplished by chemically stripping the fatty acids off the glycerol backbone of the molecule, concentrating and purifying and then reattaching to glycerol. These may be more concentrated and are absorbed well from the GI tract. However, they are rarely found in OTC or veterinary formulations because of their expense.

Ethyl esters are regularly found in human OTC products and some veterinary products. They have high concentrations of EPA and DHA but are not absorbed well from the GI tract and more prone to oxidation. This form is processed by chemically stripping the fatty acids off the glycerol backbone but then reattaching them to an ethyl alcohol backbone allowing higher concentrations to be achieved. Depending on the process, concentrations of EPA/DHA can vary from 40-90% but price becomes an issue at the higher levels. However, bioavailability is significantly lower than triglycerides and free fatty acids and may be as low as only 20-30%. Free fatty acids are rarely found in veterinary products. They have high concentrations of EPA and DHA and are well absorbed from the GI tract. In this form, the fatty acids are left free after stripping from the glycerol backbone. Therefore, they can be directly absorbed after ingestion without bile acid and enzymatic breakdown. Concentrations of EPA/DHA can be as high as 75-80% with most cost-effective products at 55-60% or double what is found in triglycerides. This concentrated form has resulted in the ability to use fewer softgels or less oil to get comparable levels of EPA and DHA.

In addition to testing for the contaminants mentioned above, fish oil products (diet or supplement) should be tested and labeled for EPA and DHA levels in order to better calculate
effective dosages. Simply reporting total amount of fish oil or total omega-3 fatty acids does not tell one about these critical components which help to support the normal inflammatory response. This is a real problem for interpreting food and supplement labels as there is no regulatory requirement to list individual omega-3 components.

**Labeling of Veterinary Omega-3 Supplements and Diets**
Label dosing recommendations for veterinary omega-3 fatty acid softgels are seldom found at levels consistent with the published evidence as described in this presentation. For some products, as much as 5-6 times the label dose would be required to reach what have been demonstrated to be effective! Therefore, it is important that EPA/DHA levels appear on the label and that one calculates a proper amount based on the patient’s body weight. Very few diets actually list EPA/DHA content on their labels or are they tested for in the finished product after heat processing. Listing total fish oil or total omega-3 levels is not the same and makes dosage calculations difficult.

**Ensuring Quality in Fatty Acid Products and other Veterinary Supplements**
The FDA regulates foods and drugs in the US so is ultimately responsible for the regulation of animal health supplements. For human supplements, there are codified regulations in the Dietary Supplement Health and Education Act. Unfortunately, the law does not apply to animal health supplements so quality of these products may be variable and unpredictable. In order to help address this issue, a non-profit trade association called the National Animal Supplement Council (NASC) (www.nasc.cc) was established in 2002. This organization is currently comprised of more than 100 member companies and works closely with FDA and AAFCO to establish fair and reasonable quality standards.

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VECTOR CONTROL IS ALSO IMPORTANT FOR CATS

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There are multiple feline flea and tick borne agents have been grown or amplified from blood, or have induced serum antibodies in the serum of normal cats or those with clinical signs like fever. As high as 80% of fleas collected from cats contain at least one organism that could induce illness in cats or people. The purpose of this review is provide an update on the diagnosis and management of feline vector borne diseases of significance. Anaplasma phagocytophilum, Bartonella spp, Borrelia spp., Ehrlichia spp., hemoplasmas, and Rickettsia spp. infections of cats will be discussed. Please also see the AAFP Panel report on feline bartonellosis www.catvets.com and the ACVIM Ehrlichia Consensus Statement (www.acvim.org) for further information.

Feline anaplasmosis. Canine anaplasmosis has been recognized for many years. Cats have been shown to be susceptible to A. phagocytophilum infection after experimental inoculation. DNA of A. phagocytophilum has been amplified from naturally exposed cats in multiple countries including Sweden, Denmark, Ireland, and the United States. The easiest way to remember the distribution is to remember the range of Ixodes spp., people with Lyme disease, and dogs with Lyme disease. Ixodes spp. transmit both A. phagocytophilum and B. burgdorferi but the current evidence suggest that A. phagocytophilum is the more likely cause of the clinical and laboratory abnormalities.

While the pathogenesis of disease associated with A. phagocytophilum in cats is unknown, some cats experimentally inoculated with A. phagocytophilum developed anti-nuclear antibodies and increased IFN-gamma mRNA suggesting that an immune pathogenesis of disease may contribute to the clinical findings. Fever, anorexia, and lethargy are the most common clinical abnormalities in naturally infected cats. Whether or not this agent is associated with chronic recurrent fever in cats is unknown.In a recent experimental study, cats infected with A. phagocytophilum by exposure to wild caught adult Ixodes scapularis from Rhode Island remained clinically normal over the 70 day study period in spite of being PCR positive for A. phagocytophilum DNA in blood for several weeks. In a larger unpublished study, we infested 10 cats with I. scapularis twice and induced A. phagocytophilum or Borrelia burgdorferi infection in all 10 cats. While repeated or new infections with both organisms occurred, all cats remained clinically normal. Since both studies were performed using ticks from the same region, it is possible a less pathogenic strain of the organism was present.

Cats with fever in endemic areas can have blood smears examined cytologically but morulae are not always detected in cats with clinical signs of anaplasmosis. Some commercial laboratories offer serologic testing or PCR assays to amplify A. phagocytophilum DNA from blood. Approximately 30% of cats with proven clinical infections induced by A. phagocytophilum are seronegative when first assessed serologically, but most of the proven cases evaluated to date
have ultimately seroconverted. Some mountain lions with *A. phagocytophilum* DNA amplified from blood have been serum antibody negative and so a single negative antibody result in an acutely infected cat does not exclude infection. Therefore, cats with suspected anaplasmosis may need convalescent serum samples to prove infection. Alternately, antibody testing could be combined with PCR testing of whole blood in acute cases. The SNAP4DX Plus (IDEXX Laboratories) has been shown to be accurate for the detection of *A. phagocytophilum* antibodies in cats. In addition, another peptide (P44-4) than the one used on the commercial assays detected antibodies even sooner.

Several antibiotics have been administered to naturally infected cats, but all cats in 3 studies became clinically normal within 24 to 48 hours after initiation of tetracycline or doxycycline administration and recurrence was not reported. While clinically normal, the organism DNA can still be amplified from the blood of some cats which suggests that treatment with tetracyclines for 21 to 30 days may be inadequate for eliminating the organism from the body. In one of our recent studies, the fact that an owner paid for a tick control product was not associated with decreased risk of having *A. phagocytophilum* antibodies in serum. These results suggest lack of compliance or lack of efficacy.

DNA homologous with *A. platys* has been amplified from the blood of cats in some countries with *Rhipicephalus sanguineus*. Further studies will be required to determine if disease associations exist with this agent in cats.

**Feline bartonellosis.** A number of *Bartonella* species including *B. henselae*, *B. clarridgeiae*, *B. koehlerae*, *B. quintana* and *B. bovis* have been cultured or amplified from client-owned cats with fever. Fever following experimental inoculation with *B. henselae* has been documented in a number of studies including a recent study in our laboratory where the CSU-1 strain of *B. henselae* induced significant fever in three of six cats after exposure to infected *C. felis*. None of the six cats administered imidacloprid-moxidectin in that study became infected or febrile. However, not all strains of *Bartonella* spp. induce fever in all cats; for example in the imidacloprid-moxidectin study, cats inoculated with the same strain intravenously failed to develop fever. Whether fever will occur during *Bartonella* spp. infection is likely a complex interaction that is influenced by both host and organism factors.

As *B. henselae*, *B. clarridgeiae*, *B. koehlerae* are transmitted by fleas, bacteremia and antibody positive rates can be very high. For example, serum antibodies were detected in 93% of cats housed in a North Carolina shelter and *Bartonella* species DNA was amplified from the blood of > 50% of cats housed in Alabama or Florida shelters. The majority of these cats were thought to be normal, which emphasizes that fever from bartonellosis cannot be documented by test results alone. In one study of pair matched cats with or without fever, serum *Bartonella* antibodies detected by ELISA or Western blot immunoassay were not correlated to the presence of fever. In addition, serum antibody test results are negative in between 3 and 15% of bacteremic cats. Thus, if a cat with fever is to be evaluated for *Bartonella* species infection the combination of blood culture or PCR assay on blood, and serologic testing will detect the greatest number of cats that are currently or previously infected. Febrile cats that are seronegative and negative for *Bartonella* spp. in blood by culture or *Bartonella* spp. DNA in blood are unlikely to have the
organism as the cause of fever. However, addition of blood culture using BAPGM media is more sensitive that routine culture (www.galaxydx.com).

Fever, lymphadenopathy, uveitis, endocarditis, myocarditis, osteomyelitis, and hyperglobulinemia appear to be the most common clinical manifestations of bartonellosis in cats. Upper respiratory tract disease, stomatitis, conjunctivitis, and pancreatitis do not seem to be associated with feline bartonellosis. If fever or other acute signs from bartonellosis is suspected in a cat, administration of doxycycline is usually effective but does not eliminate infection. The AAFP Panel Report on Bartonellosis (www.catvets.com) recommended doxycycline at 10 mg/kg, PO, daily for 7 days as the initial therapeutic trial. If a positive response is achieved, continue treatment for 2 weeks past clinical resolution of disease or for a minimum of 28 days. If a poor response is achieved by day 7 or doxycycline is not tolerated and bartonellosis is still considered a valid differential diagnosis, fluoroquinolones are appropriate second choices. In experimental or field studies, administration of enrofloxacin or orbifloxacin have led to rapid resolution of fever in cats with presumed bartonellosis. Azithromycin is now considered contraindicated because of rapid induction of resistance. Pradofloxacin (Veraflox; Bayer Animal Health) at 7.5 mg/kg, PO, once daily is considered by many to be the optimal drug for treatment of clinical bartonellosis as it is the least likely to induce resistant strains. Use of imidacloprid containing products (Advantage Multi or Seresto Collars; Bayer Animal Health) have been shown to block transmission of \textit{B. henselae} amongst research cats.

Frequent contact with animals infested with \textit{C. felis} is likely a common way for people to acquire bartonellosis and veterinarians have increased risk. Cat scratch disease has been the greatest concern over the years but is actually not the most important manifestation in veterinarians. It is now recognized that \textit{Bartonella} spp. infections of people is associated with endocarditis and many chronic inflammatory disease syndromes that can be confused with other infection or immune-mediated diseases like polyarthritis. Neuro-bartonellosis with headaches and blurred vision is common. If an animal care provider has an undiagnosed chronic inflammatory disease, they should be tested for bartonellosis. The most sensitive techniques combine culture and PCR assay (www.galaxydx.com).

**Feline ehrlichiosis.** While canine ehrlichiosis is well characterized, less is known about the agents associated with disease in cats. \textit{Ehrlichia}-like bodies or morulae have been detected in peripheral lymphocytes or monocytes of naturally exposed cats in a number of countries including the United States, Kenya, France, Brazil and Thailand. One study of cats in North America amplified DNA consistent with \textit{E. canis} from naturally infected cats. However, other studies of cats in endemic areas (Florida and Arizona) have failed to amplify \textit{Ehrlichia} spp. DNA from the blood of cats even though the agent is common in the region in dogs. In 2 separate experimental studies, we have failed to amplify monocytotropic \textit{Ehrlichia} spp. from blood or detect seroconversion in cats inoculated SQ with different strains of cultured \textit{E. canis} (Lappin and Breitschwerdt, unpublished observations, 2007; Lappin and Little, unpublished observations, 2010). These results indicate the \textit{E. canis}-like DNA amplified from naturally-infected cats may be from a different \textit{Ehrlichia} spp. more infective to cats, not all \textit{E. canis} stains will infect cats, not all cats are susceptible to infection by \textit{E. canis}, or SQ inoculation is not an effective method for infecting cats with \textit{E. canis}. Some cats with suspected clinical ehrlichiosis have seroreacted to \textit{E. canis} or \textit{N. risticii} morulae in IFA tests suggesting natural exposure.
Fever is one of the reported clinical abnormalities detected in cats with suspected ehrlichiosis and so testing may be indicated in these cats. However, a validated serological assay is not currently available and some cats with *E. canis*-like DNA in blood were seronegative. Positive serologic test results occur in both healthy and clinically ill cats, and so a diagnosis of clinical ehrlichiosis should not be based on serologic test results alone. *Ehrlichia* spp. PCR and gene sequencing can be used to confirm infection and should be considered the tests of choice at this time.

Clinical improvement after therapy with tetracycline, doxycycline or imidocarb dipropionate was reported for most cats with suspected mononcytotic ehrlichiosis. However, for some cats a positive response to therapy was a criterion for the diagnosis of ehrlichiosis. The current recommendation of the ACVIM Infectious Disease Study Group (www.acvim.org) is to administer doxycycline (10 mg/kg PO q24h or 5 mg/kg PO q12h for 28 days). For cats with treatment failure or those intolerant of doxycycline, imidocarb dipropionate can be administered (5 mg/kg IM or SQ twice, 14 days apart). Salivation and pain at the injection site are the common adverse effects and imidocarb efficacy is in question for the treatment of canine monocytic ehrlichiosis.

**Feline hemoplasmosis.** Fever or hemolytic anemia are the most common manifestations of *Mycoplasma haemofelis*, ‘*Candidatus Mycoplasma haemominutum*’, or ‘*Candidatus M. turicensis*’. In multiple studies of experimentally infected cats, *M. haemofelis* is apparently the most pathogenic species. Dual infection with hemoplasmas may potentiate pathogenesis of disease. In one study, cats with chronic ‘*Candidatus Mycoplasma haemominutum*’ infection had more severe anemia and longer duration of anemia when experimentally infected with *M. haemofelis* when compared to cats infected with *M. haemofelis* alone. In one abstract, we reported an association between *M. haemofelis* and fever in cats without anemia. Clinical signs of disease depend on the degree of anemia, the stage of infection, and the immune status of infected cats. Direct transmission may occur with the hemoplasmas and so the agents should be on the differential list for cats with a history of fighting.

Diagnosis of hemoplasmosis is based on demonstration of the organism on the surface of erythrocytes during examination of a thin blood film or by PCR assay results. Organism numbers fluctuate, so blood film examination can be falsely negative up to 50% of the time. The organism may be difficult to find cytologically, particularly in the chronic phase. Thus, PCR assays are the tests of choice due to sensitivity.

Doxycycline is often administered as a flavored suspension (to avoid esophageal strictures) at 10 mg/kg, PO, every 24 hours for a minimum of 7 - 10 days. In cats intolerant of doxycycline, enrofloxacin given at 5 mg/kg, PO, every 24 hours for 14 days was tolerated by cats and is equally effective or more effective than doxycycline. Administration of marbofloxacin or orbifloxacin gives similar results. Pradofloxacin (Veraflox; Bayer Animal Health) at 7.5 mg/kg, PO, once daily is considered the optimal drug for treatment of clinical hemoplasmosis as it is the only antibiotic shown to clear *M. hemofelis* bacteremia. However, negative PCR assay results were not achieved in all cats. Azithromycin was not effective for the treatment of hemoplasmosis in one study. Most drug protocols have failed to eliminate infection and so at
this time there is no clinical utility to repeat PCR testing. The owners should be warned that recurrences may occur but are unusual.

**Feline rickettsiosis.** *Rickettsia* spp. are obligate intracellular gram negative bacteria that are divided into the spotted fever group and the typhus group. Cats can be infected by *Rickettsia felis* and have been shown to have antibodies against *R. rickettsii*. *Rickettsia felis* DNA has been amplified from *C. felis*, *C. canis* and *Pulex irritans*; these fleas have a worldwide distribution. *Ctenocephalides felis* is a biological vector for *R. felis*; the organism can be transmitted transovarially and transtadially within the flea. Rickettsial infection is suspected to be a cause of fever in cats but this has not been well documented. While we have commonly amplified *R. felis* from *C. felis* (67.4% of flea extracts in one study), we have not amplified the organism from the blood of healthy cats or cats with fever. However, in one study of cats with fever we showed *R. felis* and *R. rickettsii* antibody prevalence rates in cats in the USA to be 5.6% and 6.6%, respectively but neither organism was amplified from blood. These results prove that cats are sometimes exposed to spotted fever group organisms but further data are needed to determine significance of diseases associations. It is now known that dogs are a more important reservoir for this agent. Because clinical illness in cats has not been documented, optimal treatment is unknown. However, based on results in dogs with *R. rickettsia* infection, doxycycline or a fluoroquinolone would be logical choices.

**Summary.** Flea and tick control is warranted for cats as well as dogs. In addition to those agents discussed previously, there are other infectious agents of cats that are vector and can be associated with illness in cats with appropriate clinical findings and geographical locale including *Coxiella burnetii*, *Cytauxzoon felis*, *Francisella tularensis*, *Hepatozoon* spp., *Leishmania* spp., and *Yersinia pestis*.

Select References


FELINE OTITIS: DIAGNOSIS AND TREATMENT

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Introduction
Feline otitis, although less common compared to dogs, is usually a multifactorial problem that can be challenging to diagnose and treat. Successful management requires an understanding of the various causes and factors and a strategic plan.

Key Points
• Feline otitis is often a diagnostic and therapeutic challenge.
• It is important to identify and control the causes and factors that contribute to otitis.
• Cats are less susceptible to secondary otic infections than dogs.
• Although some allergic cats have concurrent otitis, this is not as common as in dogs.
• Cats usually develop otitis media without overt otitis externa.
• Most often otitis media in cats occur as a sequela to respiratory disease.
• Otitis media sometimes can be a perpetuating cause of recurrent otitis externa.
• The incidence of otitis media in chronically affected ears in cats with a normal tympanic membrane is not known.
• Otitis interna has been rarely reported in cats.
• Some cats naturally have excessive waxy material in the ears and should be left alone.
• Treatment of feline otitis externa requires proper topical and oral treatments.
• Topicals should be minimized as cats are more susceptible to ototoxicity than dogs.
• Successful treatment should be determined by improvement in symptoms, otoscopy and cytology exams during recheck visits.

Understanding Feline Otitis – Etiopathogenesis
Feline otitis can be classified in primary and secondary causes and predisposing and perpetuating factors.

Primary Causes: These are usually the inciting agents that directly causes damage or disease in a normal ear canal. The cause may be subtle and go unrecognized until a secondary cause occurs. Most cases have a primary cause. The most common primary causes in cats are ear mites (Otodectes cynotis), which was reported to be responsible for up to 50% of otitis externa causes in cats. Others include hypersensitivities (i.e, atopic dermatitis, food allergy, contact allergy), other ectoparasites (Demodex sp, ticks), foreign bodies, keratinization disorders (eg. idiopathic ceruminous otitis), masses (neoplasia, polyps and ceruminous cystomatosis), upper respiratory diseases, soft palate abnormalities, autoimmune diseases (eg. pemphigus foliaceus), drug eruptions, vasculitis.

Secondary Causes: These factors cause disease in an already abnormal ear and contribute to otitis. When they are chronic or recurrent is usually because the primary cause of contributing factors have not been properly addressed. Secondary ear infections such as bacteria (e.g.
*Staphylococcus* sp) and fungus (e.g. *Malassezia, Aspergillus, Candida*). Secondary causes have also been considered perpetuating factors.

**Predisposing Factors:** These factors alone do not cause otitis but increase the risk of development and persistence of chronic infection as they facilitate inflammation by altering the ear canal microenvironment and allowing pathogenic or opportunistic organisms to establish. These factors work in conjunction with primary causes and perpetuating factors. Examples include increased environmental temperature and humidity, excessive moisture, adverse reactions to treatments and systemic diseases (e.g. FeLV or FIV, diabetes mellitus, neoplasia).

**Perpetuating Factors:** These factors are changes in the anatomy and physiology of the ear that occur in response to otitis externa even if the primary factor has been resolved. These factors may prevent resolution of otitis when treatments are only directed at primary and secondary causes. They can accentuate or permit secondary causes such as infection, by providing environments and microscopic niches that favor their persistence. These factors include ceruminous debris and concretions (e.g. ceruminoliths), otitis media, over cleaning and topical therapy (excessive moisture, maceration, physical trauma) and less commonly, proliferative changes including hyperplasia and stenosis,

**Approach to Feline Otitis**
The proper management of feline otitis requires an understanding of feline ear anatomy, which differs from the canine, and accurate investigation of the causative and contributing factors. It is important to perform a complete history, a detailed physical and otic exams along with proper diagnostic tests. It is possible to help limit the differential diagnoses based on historical findings and identification of a unilateral versus a bilateral problem. Unilateral causes include foreign body, polyps, neoplasia or trauma. Bilateral problems are usually parasitic, allergic, metabolic or autoimmune, although these can also be unilateral. Dermatophytosis, bacterial or yeast infections may be unilateral or bilateral.

Aims of Treatment: 1. Remove debris and discharge; 2. Eliminate infection from the ear canal and middle ear; 3. Reverse chronic pathological changes, if present (rare in cats); 4. Identify and treat the primary cause of otitis (most importantly).

**Important Diagnostic Procedures**
*Otic Exam:* A good quality otoscope allows the evaluation of the amount and type of exudate, presence of ear mites, estimate otic inflammation, hyperplasia and stenosis, the status of the tympanic membrane and presence of masses. Neurological exam can help identify signs of otitis media and interna, such as Horner’s syndrome, head tilt and ataxia.

*Sample for mites:* A sample of the exudate smeared onto a slide with mineral oil can be performed to look for ear mites (*Otodectes cynotis*).

*Otic Cytology:* This is an essential initial step to determine the presence of secondary infections and assist with therapy. It is important to identify inflammatory or neoplastic cells and the type of bacteria and yeast. Care should be used when collecting otic samples to avoid trauma to the ear canal and/or tympanum.
Bacterial Culture and Sensitivity (C&S): It is indicated in recurrent and refractory cases, with suspected resistance and otitis media. The correlation between C&S results and response to topical therapy does not always correspond well. Higher concentrations of topical antibiotics may prove efficacious, even when resistance has been suggested to lower antibiotic concentrations. C&S should not be done without cytology.

Imaging: Routine radiographs rarely yields useful information. Computed tomography (CT) or magnetic resonance imaging (MRI), under general anesthesia, are better to analyze the extent of the problem when otitis media, neoplasia or polyps are suspected.

Biopsy: Biopsy should be performed for definitive diagnosis when nodules or masses are present in the canal or primary causes such as auto-immune diseases are suspected. Removed otic tissues should always be sent for histopathologic analysis.

Other procedures: Because cats may have a metabolic cause for their otitis, complete blood cell count, chemistry profile, thyroid, FIV and FeLV testing may be indicated.

Specific Diagnosis and Treatment of Causes and Contributing Factors

Ear Mites: Otodectes cynotis is the most common primary cause of otitis externa in cats. Infestations are communicable and most commonly seen in young cats. Some individuals may carry large numbers of mites and have minimal signs. Others may harbor relatively small numbers of mites, but have significant otitis. Asymptomatic carriers can be a source of infestation. The exudate is usually dark-brown to black waxy, dry and granular. Secondary infections may occur. Diagnosis is usually by otoscopic examination and otic exudate examination. Treatment includes: 1. Selamectin (Revolution® top spot; Pfizer) applied between shoulders, two therapies every 3-4 weeks; 2. Advantage Multi® spot-on for cats (Bayer) two therapies every 4 weeks; 3. Fipronil (Frontline® spot on; Merial; not approved for this use in cats), one drop in each ear and rest on back, 2 therapies every 3-4 weeks; 4. Oral ivermectin (not approved for this use in cats) at 0.3 mg/kg PO once weekly for 4 weeks or subcutaneously every 10-14 days for two treatments; 5. Topical ivermectin (not approved for this use cats) 1 part injectable ivermectin (10 mg/ml) to 9 parts mineral oil or propylene glycol, once or twice weekly for 4 weeks. Proprietary approved preparations for cats include: topical ivermectin (e.g. Acarexx®, 0.01% ivermectin, Blue Ridge Pharmaceuticals) or milbemycin (MilbeMite®, Novartis) two treatments every 2-3 weeks; 6. Many pyrethrin, pyrethroid or rotenone products are available and are seemingly effective. All "in contact" individuals should be treated. If severe otitis and secondary infections are noted, products such as Tresaderm® (Merial; neomycin, thiabendazole, dexamethasone) or Otomax® (Schering; gentamicin, clotrimazole, betamethasone; not approved for use in cats) may be used.

Demodex cati: This mite may be seen when performing routine cytological or ear mite exam. Clinical signs may be mild and confined to ear canals. Large amounts of brown waxy debris may be seen. It may be seen in immunocompromised cats. There are no standard protocols for treating feline aural demodicosis. Treatment options include those suitable for ear mites. Topical ivermectin preparations may be used. If generalized disease is present, the treatment should be aimed to treat the cause of the demodicosis.
**Foreign Bodies:** This is an uncommon cause of otitis externa in cats. Although it usually presents as a unilateral problem, it may be bilateral. Most commonly, affected cats have acute onset of head shaking and ear pruritus. There is no initial discharge but secondary infections can occur if the problem is not readily identified leading to abnormal, usually purulent discharge. Examples of otic foreign bodies include plant awns and fox tail. Identification and removal of the foreign body should be curative.

**Hypersensitivity Disorders:** Food and environmental allergies should be considered in cases of recurrent or chronic otitis externa. Concurrent clinical signs including generalized skin disease and pruritus may be present; however, otitis externa may be the only sign of allergic disease. It is important to rule out other causes of otitis externa first. Secondary infections are common. A food trial should be performed in all cats with year-round symptoms prior to pursuing environmental allergies.

**Idiopathic Ceruminous Otitis:** Excessive cerumen may lead to a shiny-appearing pinna or waxy brown debris within the canals and may be associated with inflammation. Allergic diseases may be a primary cause. The use of topical and, rarely systemic glucocorticoids, may be considered. Secondary infection may occur. Some cats may present mild waxy debris without causing any problems and may not require any intervention.

**Aural Polyps:** Inflammatory polyps are the most common benign otic masses seen in cats. The etiology is unknown. Whether inflammation and infection are primary or secondary factors is unclear. They arise from the mucosal lining of the middle ear, Eustachian tube or pharynx. They may extend through the tympanic membrane into the external ear canal and result in otitis externa. In a recent post-contrast CT study including 22 cats, polyps were present in the tympanic cavity in 15 (68%) cats (three with extension into the nasopharynx), only in the nasopharynx in four (18%) cats, and only in the external ear canal in the remaining three (14%) cats. Polyps tend to occur in cats younger than 2 years of age; however, they can occur in older cats. Abyssinian cats may be overrepresented. Polyps may be acute or chronic and may go unrecognized. Polyps are usually unilateral, but can be bilateral. Clinical signs are variable and include pruritus, head shaking, abnormal exudate, inflammation, head tilt, ataxia, nystagmus, Horner's syndrome. Cats with nasopharyngeal polyps may show dysphagia, upper respiratory signs such as stertorous respiration, nasal discharge, sneezing, voice change, or dyspnea. Secondary ear infections are common and should be diagnosed and treated properly. Diagnosing ear polyps begins with signalment, history, and oral and otoscopic examinations and may require general anesthesia. Nasopharyngeal masses tend to be pink, pedunculated and have ulcerations. External ear polyps are oval to elliptical, often red, pink, or white, and glisten due to a mucosal covering. Polyps that have not extended through the tympanic membrane may distort or discolor the tympanic membrane before perforation. Imaging under general anesthesia may be needed to help diagnose and identify extension of aural polyps. Skull radiographs may be helpful; however, CT and MRI allow better evaluation of the bulla. A definitive diagnosis requires histopathology. Surgery remains the preferred choice of treatment with minimal recurrence. The most successful, when there is involvement of the tympanic cavity, is ventral bulla osteotomy (VBO) with a recurrence rate of less than 8%, while the recurrence rate with per-endoscopic trans-tymanic traction technique and simple traction is reported to be 13.5% and 30%, respectively. Potential surgical complications include temporary or permanent Horner's syndrome, vestibular...
disturbances, otitis media, hemorrhage, wound drainage, hypoglossal nerve damage, damage to auditory ossicles and vascular structures, and facial nerve paralysis; however, these are minimized with careful surgical technique. Glucocorticoids may be used to reduce inflammation and the risk of recurrence.

**Ceruminous Cystomatosis:** These benign apocrine cysts can occur in any age and are relatively common. They are single to multiple dark brown-bluish nodules present in the pinnae and ear canals. Diagnosis is based on the characteristic lesions and histopathology to rule out neoplasia. Unless they are occluding the canals, they are often tolerated and do not require therapy. Discomfort and pruritus, stenosis and secondary infections may occur and should be treated properly. Surgical excision or laser ablation can be curative.

**Proliferative and Necrotizing Otitis Externa:** This is a newly recognized, uncommon condition of uncertain etiology, mostly occurring by 4 years of age, but also reported in older cats. Males appear to be predisposed with no known breed predilections. Lesions are usually bilateral with tightly adherent golden-brown hyperkeratotic crusts overlying erythematous plaques present in the medial pinnae and external ear canals. In some cases, lesions may be limited to the ear canals. Erosion, ulceration, pain, depression and anorexia may occur. Otoscopic exam shows digitally proliferative lesions, growing in the entire length of the ear canals, without middle ear involvement. Lesions may be seen on the face, perioricularly and periorally and generalized lesions have been reported. Histopathological changes are characteristic and confirm the diagnosis. Some cases may undergo spontaneous regression. Once to twice daily topical tacrolimus 0.1%, betamethasone or hydrocortisone aceponate, and systemic prednisolone, ciclosporin, retinoids and famcyclovir have been reported to be of benefit.

**Otitis Media (OM):** OM without overt otitis externa occurs more commonly in cats than in dogs. It is usually a unilateral problem but may be bilateral. Upper respiratory disease is a common cause of OM in cats. A link between OM and soft palate abnormalities has also been reported in cats. Diagnosis may be made during otoscopic exam as the tympanic membrane may be rupture, bulging outward or contain fluid behind. A normal intact tympanum does not rule out OM. Head tilt and Horner’s syndrome may be seen. Advanced imaging (CT scan and MRI) is ideal to confirm the diagnosis. Treatment involves myringotomy and middle ear flushing. Ideally, cytology and C&S from the bulla material should be performed in order to select topical and systemic antibiotics. Several months of systemic antibiotics may be needed. Common associated infections include Staphylococcus, Streptococcus, Pasteurella and Pseudomonas. Cholesterol granuloma, a non-neoplastic OM condition in humans, has been reported in one cat.

**Neoplasia:** Neoplasia is an uncommon cause of unilateral otitis in cats. The most common include squamous cell, apocrine and sebaceous carcinomas, which tend to be aggressive. Lymphoma and fibrosarcoma involving the middle ear have been rarely reported in cats. Cats may present infection, discomfort, pruritus, bleeding and inflammation. Diagnosis is based on imaging (ideally CT scan, MRI) and histopathology. Early diagnosis and proper therapy improves disease control and prolonged survival. Prognosis and treatment are variable based on the type and extent of the tumor.
Secondary Ear infections: Bacteria and yeast infections are less common in cats, compared to dogs, but can be seen. Bacterial population was reported to be significantly higher in allergic cats than in healthy cats and cats suffering from systemic diseases. Fungal population was reported to be significantly more prominent in allergic cats and in diseased cats compared with healthy cats. Otic masses are common underlying causes.

Medications for Feline Otitis
Cats appear to be more susceptible to ototoxicity than dogs, which may be caused by their otic anatomic differences. Cats also appear to develop more contact reactions and irritation from topical medications compared to dogs. In addition, ototoxicity of most topical medications in cats is not known. Extreme care should be taken when selecting otic medications for cats. Sterile physiologic saline is the safest agent ear cleanser for cats. Iodine and chlorhexidine should be avoided. Gentamicin and dioctyl sulfosuccinate should be avoided in cases of ruptured tympanum. Alcohol and higher concentrations of acids may be irritating or cause a burning sensation in ulcerated ears. Cats should be closely monitored for ototoxicity signs (facial nerve paralysis, Horner's syndrome, vestibular disturbances, deafness) and, if occurs, medications should be discontinued.

Topical Therapy

Ear Cleaners: Ear cleaner is a controversial topic in cats; however, gentle cleaning may be done 1-2 times weekly in cats that allows the care at home, mainly when ear infections or excessive cerumen is present and prophylactically in recurrent cases. Ear cleaners include Epi-Otic®, Epi-Otic Advanced®, OticClens®, MalAcetic® otic and TrizEDTA®.

Topical Antibacterials:
Selection should be based on C&S in chronic, recurrent and resistant cases, or when rods are seen. Most topical antibacterial products also contain glucocorticoids and antifungals. Products include neomycin (Tresaderm®, Panolog®) and gentamicin (Gentocin Otic®, Otomax®, Mometamax®), polymyxin B (Surolan®) and tobramycin (Tobrex® ophthalmic). Although not labeled for cats, Easotic® (gentamicin-based) may be used when difficult to medicate due to its easy application. 25% enrofloxacin (22.7 mg/ml injectable Baytril® with sterile saline and orbifloxacin (Posatex®) may also be used, although not labeled for cats. Ideally, fluoroquinolones should be used based on C&S.

Topical Antifungals:
Antifungals are recommended for otitis associated with yeast. Clotrimazole (eg. Otomax® and Mometamax®) and 1% miconazole (Conofite®) are usually very effective. Medicated ear flushes such as TrizEDTA® with ketoconazole and MalAcetic Otic® can also be used in milder cases and for maintenance.

Topical Glucocorticoids:
There are many different types and potencies. Most of them combine antibiotics, antifungals and parasiticides. Chronic and inflamed cases may benefit from topical glucocorticoids. Glucocorticoids have antipruritic and anti-inflammatory effects, decrease exudation and swelling, cause sebaceous atrophy, decrease glandular secretions, reduce scar tissue and proliferative changes. Examples include hydrocortisone (Burotic HC®), triamcinolone (Panolog®), dexamethasone (Tresaderm®), betamethasone (Otomax®) and mometasone (Mometamax® and Posatex®).
Systemic Therapy

Systemic antibiotics: May be used in OM, and in refractory or significant proliferative otitis. The author usually recommends C&S prior to systemic antibiotic selection. Higher doses are recommended to hopefully achieve middle ear penetration. A good empiric selection include amoxicillin clavulanate acid (Clavamox®) or clindamycin (Antirobe®). Oral cephalosporins may be used; however, gastrointestinal side effects are common. High doses of enrofloxacin (Baytril®) should be avoided due to blindness reported. Marbofloxacin (Zenequin®) and orbifloxacin (Orbax®) can be used based on C&S.

Systemic antifungals: Can be used in severe cases of fungal otitis. Oral itraconazole (Sporanox®) is usually recommended at 5-10 mg/kg/day for severe yeast otitis in cats. Anorexia and vomiting may occur. Ketoconazole should be avoided due to hepatopathy.

Systemic Glucocorticoids: Glucocorticoids are indicated in inflamed and painful otitis, chronic and allergic cases. Oral prednisolone (1-2 mg/kg/day) can be used initially and then tapered to the minimum alternate day dosage that controls the symptoms. The author typically recommends oral glucocorticoids for cases of OM and polyps.

In Hospital Deep Ear flushing

Deep ear flushing, under general anesthesia, can be done to remove excessive discharge to allow visualization of foreign bodies, polyps or neoplasia, abnormal or ruptured tympanic membrane and to allow topical medications to reach their target, sampling and flushing of middle ear in cases of OM. If imaging studies are needed the same day of the flushing, they should precede the flushing. There are a variety of techniques to flush the ears. The ears should be flushed with warm sterile isotonic (0.9%) saline using a fiberoptic video-enhanced otoscopic equipment (ideally) or a 5 French polypropylene urinary catheter attached to a 20 mL syringe passed through an otoscopic cone. The use of ceruminolytics before the flush is usually not recommended in cats. Once the ear is clean, the tympanic membrane is evaluated. If the tympanic membrane is not intact, cytology and bacterial C&S is performed from the middle ear cavity. Alligator forceps and ear curettes can be used to remove larger debris and hairs that do not dislodge with flushing. If the tympanic membrane is intact but appears abnormal and OM is suspected or confirmed, a myringotomy is needed to obtain samples for cytology and C&S, and to flush the middle ear cavity. Saline is flushed into the middle ear and aspirated back. The first sample collected is used for bacterial C&S and the second for cytology. The normal tympanum usually heals in 21-35 days. Ear flushing should be performed more gently and cautiously in cats. Possible complications of these procedures include Horner's syndrome, facial nerve injury, vestibular disturbance, and deafness. These complications are more common in cats than in dogs, but usually temporary. Topical antibiotic and steroids may be infused in the middle ear. After the flush, empiric topical and systemic therapy may be prescribed based on cytology, and the treatment may be modified upon C&S results. Oral glucocorticoids and pain medication may be prescribed post-procedure to reduce discomfort and inflammation. Ear flushes may need to be repeated for more favorable outcome and ears should be re-evaluated a few weeks after.
Follow-up Visits
Recheck patient regularly, in 2-4 weeks, to assess ears and therapy response by performing an otic examination and otic cytology. This step, along with client education, is very critical to the successful management of feline otitis.

Selected References
DERMATOPHYTOSIS: DIAGNOSIS AND EFFECTIVE TREATMENT
“THIS MUCH I KNOW TO BE TRUE”

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The purpose of this seminar is to summarize the best evidence based recommendations for the
diagnosis and treatment of feline dermatophytosis. As part of the committee reviewing the
literature for an evidence based consensus paper on the treatment of dermatophytosis in small
animals, the literature was searched from 1900 to 2016 for information on this topic. The
information in this seminar is drawn from findings in >70 papers on the prevalence of disease,
>60 in vivo treatment related studies of spontaneous or experimental disease, >50 laboratory
based studies, too many to count case reports, and published abstracts. Where something is an
opinion or an interpretation it will be clearly stated.

Pathogens and Classifications
The most commonly isolated pathogen from cats is Microsporum canis, although infections with
M. persicolor, M. gypseum, and Trichophyton spp have been reported. Microsporum canis is not
part of the normal fungal flora of cats and isolation on fungal culture is compatible with true
infection, fomite carriage from a contaminated environment or object, or cross contamination of
the fungal culture plate. What is of importance to know is that pathogens are being reclassified
based upon molecular testing and that philosophy of naming fungi is now One Fungus = One
Name. For the clinician, laboratories will still report common names but over time these names
will be more widely adopted. Dermatophytes are being classified in the genus Arthoderma. The
Arthoderma otae complex includes M. canis M. furrugineum, M. equimum, and M. audouinii.

Disease Prevalence
The true prevalence of dermatophytosis in cats is unknown as this is not a reportable disease and
false positive test results are common due to fomite carriage. All prevalence studies must be read
with scrutiny to ensure test results were interpreted by clinicians that assimilated clinical signs,
test results and other confirmatory testing. A review of diagnosed skin diseases in small animals
and, in particular cats, revealed that dermatophytosis was not common and did not make the top
10 diseases of cats.

Clinical Signs
The clinical signs of dermatophytosis were directly related to the interplay between two things:
1) the pathogenesis of the infection and 2) the overall health of the cat. Variations in disease
presentation and severity within a community of cats was more dependent upon an individual’s
health than on any other factor that could be evaluated. In other words, cats with severe
widespread disease have a much more severe health problem than dermatophytosis. Important
finding from experimental infection studies using either direct application models or natural
challenge models revealed that close monitoring of cats showed that infected hairs could be
detected with a Wood’s lamp as early as 5-7 days after exposure and were culture positive. From
a clinical perspective, this means that cats are shedding infective spores onto the hair coat long before clinical signs are readily apparent. (See Wood’s lamp below).

Treatment responses and/or lack of response to treatment revealed three clinical presentations of cats that are more relevant to treatment decisions than specific dermatologic changes.

- **Simple Infection:** This group consisted of otherwise healthy cats or kittens with confirmed infections. Lesions were obvious but limited in extent. Cats responded well to a wide variety of treatment protocols and/or the disease rapidly cured without treatment.

- **Complicated Infection:** This group consisted of cats with wide spread lesions, inflammatory lesions, long-haired/matted hair coats, other illnesses (most notably upper respiratory infections), a history of prior treatment, surrender for “resistant dermatophytosis”, and/or are semi-feral or feral cats. These cats required more prolonged treatment and repeat courses of treatment to achieve mycological cure. These cats did not cure until their overall health was ‘normal’.

- **Lesions Free but Culture Positive Group:** This group of cats consisted of cats mechanically carrying spores on their hair coat (i.e. “dust mops”) or cats with very early lesions that were not easily seen but mature enough to be shedding arthrospores. Fungal culture results coupled with a re-examination under both white light and a Wood’s lamp are helpful to differentiate fomite carriers from cats with early lesions; however, fomite carriers were most often identified by a rapid change in culture status from positive to negative with topical therapy alone provided they were in a clean environment.

**Diagnostic Testing**

A review of the diagnostic tests used to confirm dermatophytosis revealed that there is no one gold standard test. All tests were flawed in one way or another. All diagnostic testing results were influenced by the skill of the examiner and/or stage of the disease. The only tests that confirmed actual invasion of hairs and/or skin by dermatophytes were direct examinations and biopsy. **Opinion:** The question is not ‘what is the gold standard’ for diagnosis but more appropriately, what test or tests does this case need to confirm the presence of an active disease in order to make an informed decision (treat, not treat, euthanize, quarantine) and what tests or tests are needed to confirm the absence of an active infection, i.e. the cat poses no risk of infection. The diagnosis of dermatophytosis is not made by a test but rather by the clinical acumen and assimilation of available information.

**Biopsy and Histological Examination of Tissue**

Biopsy and subsequent histological examination of tissue was necessary to diagnose dermatophytosis when it presented in a nodular form (mycetoma/pseudomycetoma) or mimicked an uncommon disease, e.g. pemphigus. Pathogen identification was not possible without culture or PCR testing. This finding strongly supported recommendations to include tissue culture in the diagnostic work up of nodules/tumours. Nodular forms of dermatophytosis were almost exclusively in long haired cats, most notably Persian cats.

**PCR**

PCR testing for clinicians is not universally available and no prospective studies on its use in the diagnosis of first opinion cases were published. There were numerous reports of accurate diagnosis of fungal species. PCR was found to be helpful in the diagnosis of fungal species from histological sections.
Dermoscopy
There are two reports of dermoscopy in cats and one involved the diagnosis of dermatophytosis. This tool did allow visualization of suspect infected hairs (‘comma-comma like hairs). In one study, dermoscopy identified three cats with infected hairs not yet detectable by Wood’s lamp. This tool appeared to be a useful aid in identification of hairs for direct examination and/or fungal culture.

Fungal Culture
The use of fungal cultures to diagnose dermatophytosis did not become common place until after the availability of Dermatophyte Test Medium circa 1970. Shortly after its introduction into veterinary medicine, findings similar to those in human medicine were confirmed. The colour change was suggestive, but not diagnostic, of a pathogen as many contaminants can cause a colour change. False negatives due to overgrowth, particularly contaminant fungal organisms were common. False positives due to fomite contamination and misinterpretation were also common. The early literature between 1970 and 1993 that reported on the diagnosis of dermatophytosis in cats readily recognized the problem of false positives due to fomite carriage and environmental contamination. Retrospective and prospective laboratory studies reported conflicting results regarding fungal culture findings in comparison to Wood’s lamp examinations and direct examinations. Common to these studies were reliance on outside submission of samples of varying types and quantities. Experimental infection studies using either direct application or challenge models showed agreement amount fungal culture results, direct examinations and Wood’s lamp examinations in the ‘diagnosis’ stage of examination.

Three sampling techniques were reported in the literature: combing of the hair coat with toothbrushes or carpet squares, plucking of hairs and ‘sticky tape culturing’. There are no studies of cats with spontaneous or experimental infections comparing these three techniques. Coat combings are very sensitive but did not discriminate between true infections and fomite carriage. One investigator reported 50 instances in which fungal culture was negative but infection was confirmed on direct examination.

No difference was found in difference in time to sporulation among different commercial types of DTM media. A study refuted the finding that plates need to be incubated in the dark to enhance sporulation. The most important considerations were long shelf life, easy opening of the package for inoculation and sampling and volume of medium (more is better!).

No study refuted the need to confirm species identification via both gross and microscopic morphology.

Wood’s Lamp and Direct Examinations
Review of the literature surprisingly revealed that Wood’s lamp examinations and direct examinations of hair and skin are two point of care diagnostic tools whose utility have been under-estimated in today’s clinical practice. Prior to the widespread availability of fungal culture, i.e. DTM in clinical practice, the use of a Wood’s lamp and direct examination of hairs and scales were consistently found to be useful tools. Review showed that fluorescence is common and likely an inherent property of *M. canis*. 


Review of the literature showed interesting findings regarding *M. canis* and fluorescence. It was first described in cats in 1933 and when it was used to identify an infected kitten that would have otherwise gone undetected during an investigation into the source of infection in a child. These investigators went on to examine other kittens and infect other kittens with various isolates to determine if this was a repeatable finding and it was. The use of a Wood’s lamp became a widespread diagnostic tool. Review of the literature revealed that the original source of the low percentages of fluorescence stemmed from four retrospective diagnostic laboratory studies conducted over decades where culture data, Wood’s lamp findings and direct findings were compared. No information regarding the type of Wood’s lamp, training of technicians, or number of technicians and examination procedures were reported. In vivo study findings of the commonality of fluorescence of *M. canis* published at this time were largely ignored in veterinary textbooks.

When data from spontaneous and experimental studies was examined and pooled, the following was found. Of 57 studies (n=2027 dogs and cats), information regarding Wood’s lamp findings was extractable in 30 studies. In 15 experimental infection models in *M. canis* naive cats conducted over decades, fluorescence was 100%. The statement that not all isolates fluoresce on all cats was not supported by these experimental studies. In spontaneously occurring disease (n=15 studies) when data was pooled, overall 72% of cats fluoresced. When untreated cats were evaluated, it varied from 91%-100% and in animals with previous treatment it varied from 39% to 53%. Review did not support the comment that topical therapy will remove fluorescence; investigators using baths, lime sulphur or enilconazole did not report loss of fluorescence due to topical therapy. The issue of false fluorescence was commented upon numerous times in the ‘older literature’ with recommendations to simply pluck hairs and examine the intra-follicular portion.

Reviews did support the finding that not all fluorescing hairs are culture positive during the follow-up and monitoring of response to treatment. The pigment was found to remain on the hairs even after the hair was culture negative; it is a pigment and not associated with the arthrospores or infection itself. As the infection resolves in the hair follicle, fluorescence was lost in the proximal portion of the shaft and that ‘glowing tips’ residual fluorescence was a common finding in cured cats.

Findings were similar with respect to direct examination of hair and scale, i.e. good correlation between Wood’s lamp and direct examination findings in experimental studies. When data was pooled from spontaneous infections, direct examinations were positive in approximately 61.5% of the cases. One investigator reported that for *M. canis* infections, when compared to fungal culture, Wood’s lamp examination has a positive predictive value of 90% and a negative predictive value of 94%. For direct examination, the positive and negative predictive values were 93%. Of greater interest were three studies where direct examination identified infected cats that were Wood’s lamp negative.

Opinion: Wood’s lamp examination and direct examination are useful tools in the early recognition and diagnosis of dermatophytosis in cats. Review of the past and current literature on use of the Wood’s lamp and direct examinations revealed that poor confidence in these tests may be due to inadequate equipment and diagnostic techniques.
**Technique Recommendations**

**Direct Examination:** A recent study showed that the best way to collect specimens is via both superficial skin scraping and plucking of hairs from lesions. When both techniques in cats were used unaided by a prior Wood’s lamp examination, the combined technique was positive in 87.5% of cases (Colombo 2010). The authors used mineral oil for mounting specimens. There are no studies in the veterinary literature comparing mineral oil, chlorphenolac and KOH for the detection of spores. With that said there are some practical differences to consider. The advantage of chlorphenolac and mineral oil is that both can be examined immediately or at a later time. KOH preparations require 10 to 20 minutes for digestion and need immediate examination to avoid problems with artefacts. Another problem is that KOH destroys the fluorescence on *M. canis* infected hairs making it impossible to use a Wood’s lamp to help locate glowing hairs on a slide for microscopic examination. The major advantages of mineral oil are the ready availability, no risk of injury to animals or people, i.e. KOH mistaken for mineral oil, no permanent damage to microscope lens, and no loss of fluorescence of *M. canis* hairs. The lack of digestion and clearing of epidermal scales does not affect visualization of spores and hyphae on the hairs.

**Wood’s lamp:** A description of practice tips on how to use a Wood’s lamp has been published in an open access journal (Moriello 2014 JFMS), but briefly. An electric plug in lamp with built in magnification should be used. When the author compared this lamp to other plug in lamps and battery operated lamps, the battery operated lamps were inferior and did not produce a strong beam of light and there were more false positives. In an instructional setting, students identified all positive samples when a plug in lamp with magnification was used. A key comment in the older human and veterinary literature is to hold the lamp very close to the skin to minimize fluorescence of dust etc. Move the lamp slowly over the cat and examine the entire cat but concentrate on areas that have lesions and areas where infection often starts first (face, ears, etc). Discussions with the manufacturer revealed that the lamp does not need to warm up before use; humans need to light adapt to the dark. It is very helpful to use a ‘positive’ control for reference and for training. This can be made using clear sticky tape. Press the tape over an area of strong fluorescence and then mount it on a glass slide. The edges can be sealed with clear finger nail polish. Fluorescence will last for years; the author has one 18 year old specimen.

**“CCATS” PLAN TREATMENT**
The author has previously published “CCATS” (confinement, cleaning, assessment, topical therapy, systemic therapy) to summarize treatment (Moriello 2014 JFMS, open access). Findings from review of the literature will be included in this summary.

**Confinement:** Confinement of the infected cat to an area is an important part of the treatment. Confinement makes cleaning easier and speeds time to cure because it minimizes false positive fungal culture results. When the environment of homes was monitored where cats were treated with systemic therapy and topical shampoo therapy, environments were culture negative within a week and remained so. In a review of the author’s monitoring of homes pre and post treatment or removal of infected cats/kittens, 49 of 50 homes were easily disinfected and culture negative. One home failed and that was because the owner flatly refused to comply with any cleaning recommendations and continued to bring new infected cats and kittens into the home.
Review of the literature on animal welfare, quality of life and socialization of kittens and cats requires that veterinarians reassess this recommendation and give clients very specific instructions. Dermatophytosis is most common in kittens during the critical socialization period and proper socialization of a new cat into a home is necessary so both remain in a permanent and loving home. Most cases of dermatophytosis are in cats that are new additions to the home. Confinement can be limited to what the owner would do normally when adding a new cat to a home, i.e. kitten proof space while they are at work etc. Frequent use of topical therapy will disinfect the hair coat and minimize the amount of infective material on the coat. Owners can socialize with kittens and cats using safe precaution. If children are in the home, care should be taken to educate them on how to safely play with the kitten under supervision and minimize direct contact. With early disease detection, removal of shed hairs via combing, consistent and frequent topical therapy, and systemic therapy confinement can be limited to a short period of time. Topical therapy is protective against contact with the spores especially if lime sulphur is used.

Cleaning
A careful and direct discussion about cleaning of the environment is needed for two reasons. The first is to minimize the false positive fungal culture results. Review of the literature revealed that this recommendation has been long standing. The second reason is that the disease evokes a negative response in owners disproportionate to the pathogenesis of the disease. In pet owners, this response is almost always associated with misconceptions about the environmental contamination. The biggest concern is contracting the disease from causal contact with the environment, concern about the ‘fungus living in their homes’ and concerns about respiratory illness. Review of the literature revealed that confirmed reports of transmission of the disease from a contaminated environment to a person in the absence of contact with an animal are rare.

Points to emphasize with clients:

- Fungal spores do not ‘live’ in the environment and do not multiply. They do not invade and grow in any of the home surfaces. Spores can only live in keratin.
- Fungal spores are like dust, not like mildew.
- Fungal spores are EASILY removed by mechanical cleaning and washing with a detergent and water.
- Culturing of the environment is not needed unless there is concern about false positive fungal culture results.
- These spores do not represent a respiratory risk. This is caused by overgrowth of different fungi living in the home due to excessive moisture.
- It is very likely that they or someone they live with has or had human dermatophytosis, the most common being ‘toe nail fungus.” In addition, there is no need for alarm about exposure to spores as many studies have shown people are exposed to ‘ringworm spores’ in many environments including but not limited to: their homes, other homes, gym, pools, doctor’s offices, the beach, airports, etc.
- This is a zoonotic disease but compared to other zoonotic diseases it is not life threatening or life changing.
With regard to disinfection practices, studies have shown that if it can be washed it can be decontaminated. Laundry should be washed twice or until all visible hair has been removed. Bleach and hot water were not found to be better than cold water. The most important part of the washing was long agitation times and not ‘over stuffing’ the washing machine. Carpets can be disinfected. Vacuuming should be used to remove cat hair. The carpets can be disinfected using hot water extraction, repeated washing with carpet shampooer or if necessary after pre-treatment with a disinfectant. Hard surfaces are disinfected by mechanical removal of debris, washing of the surface with a detergent until visibly clean, rinsing and removal of standing water. Disinfectants are only needed to kill spores remaining after the hard clean. If there are only one or two infected pets and a topical antifungal treatment is being used, owners can do a hard clean/disinfectant application once or twice a week provided they do adequate mechanical removal of debris followed by a one-step cleaner with antifungal effective against *Trichophyton mentagrophytes* on the label. Accelerated hydrogen peroxide products are as effective as bleach and are recommended over house hold bleach.

**Assessment-What is the response to treatment?**

Review of the literature shows that monitoring for a clinical response to treatment has always been the primary first goal. Lack of clinical response was an indicator of treatment failure for any number of reasons. Consistently clinical response preceded mycological cure. Prior to the wide availability of fungal cultures, the most common method to monitor response to treatment was via a Wood’s lamp examination. Detailed reports of the development and resolution of infections are consistent. During the early stages of disease, the proximal part of the hair shaft showed fluorescence. As the infection progress the entire shaft develops fluorescence and with eradication of the infection, it is lost in the proximal part of the hair shaft. As the cure progresses and the hair recovers, hair shaft fluorescence proceeds up the shaft until only the tips glow. These findings have been confirmed in experimental infection studies and field studies.

The term ‘mycological cure’ with respect to the treatment of dermatophytosis in cats did not appear in the literature until 1959 when two investigators reported on the use of griseofulvin for the treatment of dermatophytosis in cats. In that study, mycological cure was defined as two negative cultures taken two weeks apart. This was not based on any study or data. In addition to fungal culture status, monitoring of the number of colonies on the plate was first reported to monitor cats in 1968. Successful treatment was associated with a rapid decrease in the number of colonies on the plate and resolution of lesions.

Monitoring of response to treatment in pet cats involves: examination for the presence or absence of lesions, examination with a Wood’s lamp (assuming fluorescing hairs were found and one has an appropriate Wood’s lamp), and fungal culture results coupled with the numbers of colony forming units per plate.

**Topical Therapy**

Review of the literature revealed that miconazole/chlorhexidine shampoo, lime sulphur and enilconazole were the most commonly used topical therapies. Recently two in vivo studies were published documenting that essential oils were effective. A neutral shampoo with added essential oils of *Thymus serpillum*, *Origanum vulgare*, *Rosmarinus officinalis* was compared to a miconazole/chlorhexidine formulation. Both were effective for topical therapy. A recent in vitro
study documented that shampoo formulations containing ketoconazole, miconazole, miconazole/chlorhexidine, climbazole and an accelerated hydrogen peroxide rinse (3.5% diluted 1:10) were fungicidal against *Trichophyton* and *M. canis*.

Topical therapy recommendations include: combing of the hair coat prior to treatment to remove broken and fragile hairs, and whole body shampoo or rinses two to three times a week. The most common cause of persistently positive fungal cultures in cats that are clinically recovered but still culture positive are spores on the hair coat and/or infected hairs in difficult to treat areas. Clients are resistant to treat the face and ears of cats. Use of an otic preparation in the ears formulated for *Malassezia* is recommended daily. Products that contain ≥ 1% clotrimazole, ketoconazole, or miconazole can be used. For lesions on the face and/or around the eyes, vaginal miconazole can be applied. The ocular safety of this has been shown and it is widely used in equine medicine.

There were no studies directly addressing clipping of the hair coat but it was found that whole body clipping can spread the infection to other areas. Clipping or plucking of glowing hairs can be helpful when isolated hairs are found or to debulk the hair coat. However, in most studies this was not performed. From an animal welfare safety and to minimize contamination of the environment, scissor clipping is the recommended method to clip cats. Children’s round tipped metal scissors are ideal. They are inexpensive so they can be discarded and are blunted so there is minimal risk of injury. The hair coat was not clipped in shelter studies; clipping may not be needed in most cases if through application/drenching of topical solution can be applied.

**Systemic Therapy**

Review of the literature revealed that itraconazole, ketoconazole, griseofulvin, and terbinafine where the most commonly used systemic antifungal. Fluconazole was rarely used. No deaths were reported with the use of any of these drugs which was surprising. Both itraconazole and terbinafine have been shown to have residual activity in the hair and to be effective in a 21 days continuous treatment protocol with adjuvant topical therapy. Systemic therapy pharmacokinetic studies are performed in healthy adult cats. Pharmacokinetics will be different in cats with complicated infections; adjust treatment to fit the patient.

Eleven studies were reported on the use of itraconazole in cats. With respect to itraconazole, there were no published case reports of cats being treated for dermatophytosis that developed liver toxicity and died. The cases of liver toxicity in cats receiving itraconazole were being treated for intermediate or deep mycoses. Itraconazole is the drug of choice as it is licensed for use in cats. One of the most commonly used treatment protocols is itraconazole 5 mg/kg one week on/week off for 6 weeks (i.e. 21 days total of itraconazole). Treatment should be continued until the cat is clinically and mycological cured, assuming fungal cultures are possible.
DIAGNOSTIC APPROACH TO PRURITUS IN CATS

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Introduction
Many dermatoses of cats are associated with pruritus. The approach to feline pruritus should involve a careful systematic approach to identify the underlying disease. Clinicians often face several challenges when diagnosing a cat with pruritic skin diseases:

- To determine if the cat is pruritic (as some cats are presented only for hair loss)
- To determine the cause of the pruritus, which can be more difficult in cats than dogs
- In most cases, various diagnostic steps and treatment trials are needed to identify the etiology and successfully manage the pruritus

Etiology
When dealing with a pruritic cat, veterinarians tend to diagnose the reaction pattern or clinical manifestation of the pruritus (for example, eosinophilic granuloma complex) rather than the true etiology. It is very important to determine the cause of the pruritus. Various skin diseases can cause pruritus in cats including: hypersensitivity disorders (i.e. flea bite allergy, atopic dermatitis, food allergy, and contact allergy), parasitic disorders (i.e. surface demodicosis, notoedric mange, cheyletiellosis, otoacariosis, trombiculosis, and pediculosis), dermatophytosis, herpes virus dermatitis, thymoma-associated exfoliative dermatosis and cutaneous T-cell lymphoma. Hypersensitivities and most parasitic diseases in cats are consistently pruritic; however, pruritus is not always present in the other disorders. In addition, pruritus level may vary. Secondary superficial pyoderma, Malassezia dermatitis and otitis externa are less common in cats compared to dogs and are uncommon primary causes of pruritus, however; they may be associated with underlying diseases and can contribute to pruritus.

Signalment
It is important to review the signalment. Young cats are more likely to have parasitic diseases or dermatophytosis, while young- to middle-age adults are more likely to be allergic. In older cats, with no prior history of skin disease neoplastic or paraneoplastic dermatoses (eg. thymoma-associated exfoliative dermatitis, cutaneous lymphoma) need to be considered. Burmese and Siamese breeds tend to be more anxious and have more psychogenic contribution to their pruritus. Siamese cats may be more prone to food allergy. Persian and Himalayan cats are more likely to have dermatophytosis.

General History
A detailed and thorough history is key in helping streamline the differential diagnosis. History should include the age of onset of pruritus and/or hair loss, course and symptoms of the disease, seasonality of the problem, previous skin and/or ear diseases, life style, diet, environment, cat's temperament (anxious, hyperactive or calm) due to psychogenic factors, other animals (affected and non-affected) in the household, affected humans, concurrent diseases or symptoms and any current or previous medications used and response to therapy. Getting a copy of previous
medical records can be very helpful as owners often do not remember all details. If pruritus is observed, have owners grade pruritus on a scale of 1–10 (1 - occasional overgrooming/itching, 10 - constant overgrooming/itching) or similar scale. If more than one cat (or dog and human) is affected in the household, indicating contagion, consider contagious or zoonotic conditions such as dermatophytosis, cheyletiellosis, notoedric mange, surface demodicosis, otoacariosis, or pediculosis (the latter three diseases do not affect people and, surface demodicosis caused by *D. gatoi* and feline pediculosis do not affect dogs).

**Determining if the cat is pruritic**

It is helpful in making a diagnosis if it can be determined whether the cat has been losing hair due to overgrooming or reported itching; however, sometimes pruritus can be very difficult to recognize in cats, and is commonly misinterpreted as self-grooming. Also, some cats may be doing this in private and owners may be unaware that the cat is overgrooming or showing other signs of pruritus (i.e., "closet groomers"), mainly in those cats presenting symmetrical alopecia.

A thorough history and complete general and dermatological physical examination can be quite helpful in determining the presence of pruritus. It is important not to ask, however; the general question – "Is your cat itchy?" because some of the cat’s responses to a pruritic stimulus may not be recognized by the owner as such. Instead it should be asked if the cat licks excessively, chews, scratches, and/or constantly rubs the body against objects. Other signs of pruritus in cats include tufts of hair in the cat's favorite resting or hiding places, hairs in the teeth or tongue, frequent vomiting of hair balls, and hair in the feces. If the owner witnesses the cat itching or overgrooming, the clinician should proceed to the next challenge, which is to determine what is causing the pruritus. However, if the owner either answers “no” or “I do not know” to the question, the clinician is still faced with the first challenge of figuring out if the cat is pruritic or not.

A simple and rapid test veterinarians can pursue to determine if the cat is itchy is trichoscopy (also known as trichoscopy or trichogram). This test involves examining hairs plucked from within the affected areas under the microscope with a few drops of mineral oil and looking for evidence of traumatic changes such as fragmented hair shaft body and/or tips. As a last resort, application of an E-collar for 1 month will lead to regrowth of hair and resolution of lesions in cases of self-induced alopecia.

**The distribution of pruritus**

The distribution of pruritus can help the clinician to some degree. For example, if the owner reports that the cat scratches primarily at its dorsum, flea bite allergy (mostly caudal aspect), cheyletiellosis (anywhere along the dorsum) and, pediculosis should be considered as possible differentials.

**Clinical Signs**

A complete general and dermatologic examination, including an otic and oral exam, should be performed in all cats. The main clinical signs of the various feline pruritic diseases are not very useful diagnostic tools *per se* because they are non-specific for any particular disease. In fact, the same disease may have varied presentation in different animals. However, the distribution of lesions and the presence of external parasites (e.g. fleas, lice) can be helpful in formulating a list
of differentials and, sometimes, can be diagnostic (e.g. lice, chigger, etc.). The presence of non-cutaneous signs can also be useful (e.g., if the cat has gastrointestinal signs consider food allergy; if the cat has fever, lethargy, or decreased appetite, consider internal or neoplastic disease; if the cat has history of upper respiratory signs consider viral disease such as herpes virus dermatitis).

**Pattern analysis and recognition of relevant lesions**

This is a necessary approach for developing objective differentials. The clinical signs (also referred to as reaction patterns) of pruritic disorders of cats are variable and typically include one or more of the following:

- Miliary dermatitis
- Self-inflicted non-inflammatory alopecia
- Eosinophilic granuloma complex
- Face, head and neck pruritus
- Scaling or exfoliation

Unfortunately, these clinical presentations are not pathognomonic for any pruritic skin disease which makes it more difficult for the clinician to determine the pruritic cause. In addition, the fact that cats may have more than one of these presentations at the same time can complicate the process. Allergic skin diseases are the most common causes for these patterns; however, to date, there is no clear evidence that one pattern is more likely to represent feline atopic disease compared to another. Although feline atopic dermatitis is recognized as a common cause of pruritus in cats, it remains incompletely characterized with a varied spectrum of lesions affecting a range of body areas.

**Miliary dermatitis** is characterized by small erythematous papules, typically covered with brownish crusts. It is often associated with self-inflicted alopecia. It can be present in various pruritic diseases including flea bite allergy, atopic dermatitis, food allergy, mosquito bite allergy, cheyletiellosis, pediculosis, trombiculosis, and dermatophytosis. Flea bite allergy is the most common cause of miliary dermatitis in cats and the characteristic papulocrustous lesions are typically abundant and present along the dorsal-lumbar region/tail base. Miliary dermatitis is also a valid criterion for diagnosis of environmental- and food-induced allergies, especially when lesions are not dorsally distributed. Miliary dermatitis is not consistently associated with the other pruritic diseases and the lesions range from few to several with variable distribution.

**Self-inflicted non-inflammatory alopecia** is characterized by symmetrical alopecia that more often develops in areas where the cat can easily reach such as, the ventral abdomen, groin, lateral trunk, and legs. The associated skin is visually not inflamed but, occasionally, very few erythematous papules, scattered throughout the affected areas, are noted. Cats induce the alopecia by overgrooming, chewing, and/or pulling out groups of hairs. Owners typically do not witness the cats doing these activities, thus, the clinician will need to perform a trichoscopy to confirm the self-induced problem. The most common pruritic diseases causing this pattern include surface demodicosis (caused by *D. gatoi*) and allergies. In one study, symmetrical alopecia localized on abdomen appeared to be highly specific to feline non-flea allergic skin diseases, while on the dorsum was more characteristic of flea bite allergy. This pattern is a common presentation of behavior disorders; therefore, psychogenic causes should be in the differential diagnoses list for cats with this pattern of alopecia.
**Eosinophilic granuloma complex** encompasses three cutaneous reaction patterns:

1. **Eosinophilic plaques**: characterized by well-demarcated, eroded to ulcerated, erythematous plaques that are typically present on the ventral abdomen and medial thighs but, may occur anywhere.
2. **Eosinophilic granulomas**: typically present as well-demarcated, firm, red to yellowish, raised and often linear to nodular lesions. Lesions are frequently present on the caudal aspects of the hind limbs, but they can also occur in the oral cavity, lower lips (“pouting lips”), chin (“fat chin”), interdigital aspect of feet, and elsewhere.
3. **Indolent ulcers**: typically occur on the upper lips and are characterized by well-demarcated, erythematous ulcers with raised borders. More than one lesion may develop in a single cat.

Despite the differences in clinical presentations, the triggering causes are similar and include: food allergy, atopic dermatitis and flea bite allergy. It can also be idiopathic.

**Face, head and neck pruritus** are typically associated with excoriations, self-induced alopecia, erythema, crusts, erosions, and/or ulcerations. The types of lesions will vary according to the pruritus severity. Pruritic dermatoses that can induce this pattern include: hypersensitivities (most commonly food allergy but also atopic dermatitis, flea bite allergy, and mosquito bite allergy), parasitic disorders (i.e. otoacariosis, notoedres, and trombiculosis), and infectious (i.e. dermatophytosis, and herpes virus dermatitis).

**Scaling** can be the main clinical presentation of some pruritic skin diseases of cats. Cheyletiellosis is the most important differential diagnosis and the scales are typically present along the dorsum. Pediculosis can also present with scaling along the dorsum. Thymoma-associated exfoliated dermatosis and cutaneous lymphoma are also scaly skin disorders that sometimes may be associated with pruritus.

As probably already noticed, the same disease is mentioned as differential diagnosis for many of the clinical presentations, indicating that they are not specific of any pruritic disorder. Various aspects of the history and physical findings will help the clinician to formulate a sensible list of possible differential diagnoses. In many cases, a number of diagnostic tests will also be required before a definitive diagnosis can be established.

**Secondary Infections.**
It is important to identify infections and manage them appropriately. Bacterial (*Staphylococcus* spp.) and yeast (*Malassezia* spp.) skin and ear infections can play a significant role in feline pruritus, mainly as secondary problems. Bacterial infections are a common complication of the eosinophilic granuloma complex, and yeast infections can affect skin fold areas, the ears, and nail folds. Malassezia dermatitis is more commonly seen in allergic cats and often contributes to pruritus; however, generalized or severe Malassezia infection/overgrowth in cats may be a sign of internal or neoplastic disease.

**Diagnostic Procedures.**
The selection of the diagnostic test(s) to perform should be made after a sensible list of differential diagnoses has been formulated based on the cat’s history and clinical findings. A systematic diagnostic approach should include the following steps:
Investigate Infection(s).
The diagnosis of the underlying disease may be missed if secondary infections are not identified and controlled.

- **Cytology**: main diagnostic test used to identify yeast and bacterial infections/overgrowth. Impression smears and tape preparations can be used.
- **Bacterial culture**: should be performed when there is no response to appropriate antibiotic therapy with known good compliance, when the cat has been treated with multiple antibiotic courses or has been recently hospitalized, in the presence of deep pyoderma lesions, if rods (not suspected to be oral flora) are seen on cytology, or with a history of multi-drug or methicillin-resistant Staphylococcus infection.

Investigate Ectoparasites.
Ectoparasites and insect induced allergic dermatoses are common in cats and may be very difficult to prove in many cases. Ruling out fleas is usually the first step in the investigation of feline pruritus. Most clients assume that because they have never seen fleas on their pet, flea bite hypersensitivity cannot be involved. However, cats are fastidious groomers and tend to remove evidence of flea infestation. Therefore, trial therapy is required in most cases, even if there is no evidence of flea infestation.

- **Flea comb test**: may be useful to help confirm the presence of fleas and support a diagnosis of flea bite hypersensitivity.
- **Wet paper test for fleas**: Run a piece of white paper under the tap and then hold it next to/under the cat while you ruffle the fur all over. Flea feces are small black bits of dirt, but the contained blood means they become red streaks when wet.
- **Flea allergen intradermal test**: provides an immediate response with a wheal and flare reaction in over 75% of the cats. A delayed reaction may be observed in some cats. The evidence promotes the acceptance of the disease and often motivates the cat owner that does not accept the "flea allergy theory".
- **Skin Scrapings**: Multiple skin scrapings should be performed in every cat presenting with pruritus and alopecia. Remember that negative scrapings do not rule surface demodicosis (D. gatoi) and cheyletiellosis.
- **Hair plucks and Tape prep**: These tests may be used to assist with investigation of mites such as D. gatoi, Cheyletiella sp, chigger and lice.
- **Fecal floatation test**: This test can be useful to identify surface mites, particularly D. gatoi and Cheyletiella sp, when skin scrapings are negative, as the grooming habits allow the mites to be ingested; however, a negative test does not rule them out.
- **Parasiticidal trials**: Trial therapy involves treating the patient and all in-contact animals with parasiticides and the environment by vacuum cleaning, bed washing and spraying the environment with insecticides/parasiticides.
- **Treatment trial for fleas**: A 6-8 weeks trial should be performed on 2-week intervals. Parasiticides include fipronil (Frontline Plus, Merial), imidacloprid, selamectin (Revolution, Pfizer), Vectra for Cats, imidacloprid + moxidectin (Advantage Multi, Bayer), +/- a few days course of nitenpyram (Capstar®, Merial). Year round preventative treatment is necessary, particularly for cats in endemic areas.
- **Treatment trial for mites**: Weekly 2–3% lime sulfur sprays/rinses may be used for its parasiticidal, antifungal and antipruritic effects for D. gatoi and Notoedres sp. Selamectin
(Revolution, Pfizer) every 2 weeks for 4 doses can be used when suspecting of Cheyletiella sp, Notoedres sp and Otodectes sp.

Investigate Dermatophytosis.
Dermatophytosis is uncommonly associated with pruritus in cats; nevertheless, some cats may present with mild to severe pruritus and a work-up should be performed.
- **Trichoscopy:** Can be easily done to help identify the presence of dermatophytic arthrospores on hair shafts; however, a negative test does not rule dermatophytosis.
- **Fungal culture:** Provides a definitive diagnosis and identify the fungal organism.
- **Woods lamp examination:** Not often helpful as not all species of ringworm will fluoresce (around 50% of Microsporum canis fluoresce, other species do not) and it is possible to get false positives with bacteria or topical medications.

Investigate Allergic Diseases.
The most common hypersensitivity disorders (HD) are usually caused by environmental, food and/or flea allergens. Others less common include mosquito bite hypersensitivity and contact dermatitis. As reported for dogs, there are no well-defined criteria to establish the diagnosis of feline atopic dermatitis (FAD, or non-flea, non-food allergic dermatitis). Therefore, FAD should be considered a diagnosis of exclusion, ruling out parasites and food allergy. Every cat with non-seasonal allergic skin disease should undergo a strict elimination diet trial to rule out food allergies.

- **Elimination Diet Trial:** Having ruled out parasitic and infectious causes of pruritus, the next step is to investigate whether an adverse food reaction (‘food allergy’) is involved. Currently, the only way of truly diagnosing an adverse food reaction is by performing an elimination diet trial, as both intradermal and serologic food allergy test results have been inaccurate. This trial involves feeding strictly a novel protein and carbohydrate or a hydrolyzed diet for a period of 6-8 weeks. Diet selection for the elimination dietary trial can be made after careful review of the history. Hydrolyzed diets should be selected for those patients when a complete dietary history is unknown such as rescue pets and when the list of diets is too extensive to be accurate. Purina HA (hydrolyzed) and Feline Green Pea and Rabbit or Duck (Innovative Veterinary Diets) are usually very palatable. Rayne Nutrition diets may also be used. It is important to avoid over-the-counter foods as cross contaminations are more likely to occur. Feeding a home-prepared diet with a novel protein is believed to be, by many dermatologists, the gold standard for ruling out food allergy in cats; however, this process has become increasingly difficult as the availability of novel proteins diminishes and feeding a home-prepared diet is also expensive, time-consuming, and may be nutritionally imbalanced if that is not addressed properly. There is no single commercially available diet that effectively identifies 100% of the food allergic cats. Failure to improve on a commercially-available diet may warrant trying another one, or a home-cooked diet. Upon significant improvement or resolution of clinical signs, the animal should be challenged for 2 weeks with its regular diet to confirm the diagnosis. Recurrence of clinical signs is usually noted within two weeks. Signs should resolve when the offending food is removed.

Once all parasitic and infectious diseases have been eliminated and a diet trial has been performed, you can further investigate FAD, or treat the cat symptomatically.
• **Glucocorticoid Trial:** If allergies is strongly suspected and the cat is very itchy, a short course of anti-inflammatory doses of oral steroids (e.g., prednisolone) may be used to evaluate response and to also provide symptomatic relief; however, interpretation of response may be difficult in cats with no reported pruritus or skin lesions. Also, glucocorticoids may cause a calming effect in some cats affecting their grooming behavior, regardless of the cause.

• **Allergy testing:** Despite the uncertainty surrounding the role of IgE in FAD, dermatologists still perform allergy intradermal and/or serum. Although positive reactions are often seen with serology test, the precise significance is not well known. Intradermal testing results may be unrewarding in cats and more difficult to interpret than in dogs. Fluorescein may be injected prior to performing the test to allow reactions to fluoresce and be seen under UV light. A different form of allergy skin test typically used in humans, called prick test, has been investigated in a few healthy cats and may be good to identify positive reactions in atopic cats, however; further studies are needed before its routine use. Remember that allergy testing should be performed only after all other pruritic skin diseases have been ruled out and FAD is diagnosed. Allergy testing should be used to identify specific environmental allergies for possible avoidance and to formulate allergen specific immunotherapy and should not be used to make a diagnosis. At this time, skin and serum allergy testing for food allergens is likely to be of little or no value, as it is in dogs.

Further Dermatology Work-Up: Always perform the basic diagnostic tests first. Consider health screening blood tests, particularly if the cat is sick, has lost weight or there is suspicion for internal or neoplastic disease.

Skin Biopsy: Often not needed in feline pruritic conditions; however, it may be useful if the clinical presentation is unusual, to rule in/out neoplastic conditions, and if there is no response to rational therapy. Biopsy is a frustrating tool if used on all skin cases. Superficial perivascular dermatitis is a common response to many diseases, such as parasites, infection or allergy. However, skin biopsy can sometimes be diagnostic or at least give the class of disease you are dealing with. Multiple samples should be taken and preferably, the samples should be sent to a dermatopathologist.

Summary
Cats can have many pruritic skin diseases and, establishing the primary cause can be very challenging, more so than in dogs. A thorough history and complete physical examination can provide important diagnostic clues. Diagnostic tests are often needed and the selection of which tests to perform should be made only after a sensible list of differential diagnoses has been formulated based on the cat’s history and clinical signs. After the cause of pruritus has been identified, appropriate and systematic management of the underlying disease is key in achieving a successful treatment response.

Selected References
MANAGEMENT OF THE ALLERGIC CAT

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Introduction

In 2015, the International Committee on Allergic Diseases of Animals (ICADA; www.icada.org) updated the guidelines for treatment of atopic dermatitis (AD) in dogs. These recommendations are generally made from evidence derived from previously published randomised controlled trials and systematic reviews. No such document exists for the treatment of allergies in cats. Evidence is predominantly limited to uncontrolled clinical trials or a consensus among authors for recommending a treatment intervention.

Using the framework of these published guidelines, this lecture is divided into three different sections: recommendations for i) the management of acute flares of feline allergic skin disease, ii) the treatment of chronic skin lesions of feline allergic skin disease, and iii) the interventions to prevent disease relapses. These recommendations must be evaluated by veterinarians taking into consideration both patient and owner factors. Practitioners should always assess the benefit, side effects, practicability, cost and availability of the proposed treatments, which often will have to be combined for an optimal outcome.

ICADA has also considered the terminology for allergic skin diseases of cats. The consensus of the committee was to adopt the term “feline atopic syndrome” (FAS) which would specifically exclude parasitic causes but include environmental allergen causes and some manifestations of food reactions. The committee acknowledge that further refinement and standardisation is indicated for the terminology for feline allergic skin diseases.

1. Management of acute flares of feline allergic skin disease

   A. Identify and avoid flare factors: acute flares of FAS can be caused by flea bites; a recent increased exposure to environment allergens (especially house dust mites and pollens) and the ingestion of food ingredients. Cats with allergic dermatitis are predisposed to secondary infections with bacteria and yeast and yeast otitis externa is a common complication.

      i.) Implement flea control: definitive evidence of flea infestation can be challenging in cats reflecting both grooming activity and prompt removal of fleas. Implementing flea control with a product that rapidly reduces flea feeding is important. Response to flea control is the best confirmation that fleas are a relevant flare factor. Useful products include spinosad (Comfortis®) 50 to 100mg/kg PO q 14 days; nitempyram (Capstar®) 1mg/kg PO q 24hrs or indoxacarb (Activyl®) applied every 14 days.

      ii.) Evaluate the use of antimicrobial therapy: bacterial and yeast infections should be confirmed with cytological evaluation of skin and the external ear canal. Antimicrobial therapy is indicated, and in cats, injectable and oral antimicrobial therapies are more useful than topical medications, except for auroral preparations. Veterinarians are advised to follow antimicrobial treatment guidelines established in their country of practice and/or in international consensus recommendations.
B. Improvement of skin and coat hygiene and care
   i.) **Bathing with a non-irritating shampoo:** in allergic cats, shampoo therapy is not often used, however, if cats will tolerate bathing then it can be a useful intervention for both physical removal of surface allergens and microbes and improved skin hydration. Emollient formulations containing either lipids, complex sugars and antiseptics (Allermyl®) or phytosphingosine, raspberry oil and lipids (Douxo Calm®) have been shown to provide a modest effect on skin lesions and pruritus in dogs with mild AD. The intensity and frequency of bathing may be the most important factor in relieving pruritus. Wiping the patient with a damp cloth on a daily basis is an alternative to bathing that is often more practical in cats.

C. Reduction of pruritus and skin lesions
   i.) **Short-term treatment with topical glucocorticoids:** topical glucocorticoid sprays (Cortavance®, Genesil®) are effective for treatment of flares of canine AD. Topical daily application of 0.0584% hydrocortisone aceponate (HCA) spray (Cortavance®) is effective for reduction of skin lesions and pruritus in allergic cats. In cats that do not tolerate the application of the spray, the product can be wiped on lesional skin using cotton wool. Other medium potency topical corticosteroids that can be useful for local topical application include 0.1% mometasone or 0.1% methylprednisolone aceponate lotion. None of these products are registered for use in cats and caution should be exercised to avoid ingestion by grooming.

   ii.) **Short course of oral glucocorticoids:** oral prednisolone or methylprednisolone is typically dosed orally 1–2mg/kg per day to initiate therapy, then tapered to 0.5–0.1mg/kg every 48 hours for maintenance; dosing for glucocorticoids is generally higher in cats compared to dogs to achieve the same clinical effect. Adverse effects of oral glucocorticoids are normally proportional to drug potency, dosage and duration of administration. Treatment with long-acting injectable glucocorticoids is not recommended.

   iii.) Short course of oclacitinib: oclacitinib (Apoquel ®) at 0.4–0.6 mg/kg orally twice daily for up to 14 days rapidly reduces skin lesions and pruritus in dogs with AD and short-term treatment appears safe. Limited published studies are available reporting the pharmacokinetics or clinical use of JAK inhibitors in cats and the drug is not registered for use in this species. Oclacitinib administered at 0.4–0.6 mg/kg q 12hrs for 14 days tapering to q 24hrs for 14 days improved pruritus scores in less than 50% of 12 cats in a small pilot study. There may be a potential role for oclacitinib in the treatment of feline allergic dermatitis but prospective studies in cats are needed before this medication can be recommended for clinical use.

2. Treatment of chronic feline allergic dermatitis
   A. Identify and avoid flare factors
      i.) **Implement a flea control regimen:** in geographic regions where flea infestation is endemic, all cats with allergies should be treated with year-round flea adulticides combined with relevant environmental measures. Insecticides that demonstrate long effect and fast residual speed of kill are more effective in flea allergic cats.

      ii.) **Evaluate use of antimicrobial therapy:** topical and/or systemic antimicrobial therapy is indicated when a skin and/or ear infection with bacteria and/or yeast is diagnosed based on compatible clinical signs with or without supportive cytology or bacterial culture.
Terbinafine or itraconazole can be prescribed once daily or for two consecutive days each week for three weeks to treat flares provoked or exacerbated by *Malassezia* skin infections in dogs with canine AD. The author has found this a useful strategy for in Sphinx and Devon Rex cats with FAS with relapsing *Malassezia* dermatitis.

iii.) Perform a restriction diet trial in cats with non-seasonal pruritus: in dogs, as in humans, food allergy can manifest with clinical signs of AD. Food allergens can cause flares of clinical signs of FAS in cats. The current gold standard for the diagnosis of adverse food reactions remains a restriction trial with novel and/or hydrolysed diets followed by provocation with original food items once signs have abated during the restriction phase. An 8-week restriction-provocation dietary trial should permit the diagnosis of an adverse food reaction in a cat.

It is speculated that the presence of storage mites in dry dog foods might cause some relapses of canine AD because of their allergenic cross-reactivity with house dust mites. Freezing dry dog foods might reduce contamination with storage mites, but the impact of such freezing on the clinical signs of mite-hypersensitive dogs is unknown. To decrease excessive storage mite contamination, owners should be encouraged to avoid storing commercial dry dog foods in humid and warm areas, and they should be advised to store foods in clean and sealed containers. While there is no published evidence of the role of storage mites in dry cat foods, the same strategies are recommended for cats with FAS that are positive on allergy testing for house dust mite.

iv.) Intradermal and/or IgE serological allergy testing: allergen-specific intradermal testing (IDT) and/or IgE serologies identify environmental allergens in cats with FAS. Positive immediate IDT reactions and IgE serology to environmental allergens can also be observed in cats without signs of FAS. As a result, these tests cannot be used to differentiate cats with FAS from healthy cats or cats with other pruritic dermatoses.

B. Improve skin and coat hygiene and care

i.) Bathing with a non-irritating shampoo: if owners can bath cats with minimal stress, then weekly bathing with a mild non-irritating shampoo and lukewarm water is likely to be beneficial for a direct soothing effect to the skin, the physical removal of surface allergens and microbes and an increase in skin hydration.

ii.) Supplementation with oral EFAs: the benefits of EFA supplementation have been evaluated in several older studies on cats with allergic skin disease. Most of the studies, however, were not controlled nor randomised with unclear inclusion and exclusion criteria. Although the responses were variable among all of the studies, clinical improvement was reported for most cats with regard to lesion resolution and pruritus reduction although statistical significance was questionable. Whether clinical improvement from EFA supplementation in cats with FAS is related to improved barrier function, the anti-inflammatory effects of fatty acids, or simply an improved quality of overall hair coat is uncertain. The relative safety of EFA supplementation makes this option appealing as part of the management strategy, particularly in the mildly affected allergic cat.
At this time, there is no evidence of superiority for any particular EFA combination, dosage, ratio or formulation (including enriched diets) to improve skin and coat quality in cats with FAS. In general, EFA-enriched diets provide higher amounts of EFAs than oral administration of EFA supplements. Various dosing formulations exist, which may be added to the food (capsule, oil, spray); efficacy should be assessed after at least 6 to 8 weeks of administration. Side effects of EFA are uncommon but palatability for cats is often poor.

iii.) Application of topical EFA-containing formulations: topical lipid formulations can normalise existing stratum corneum lipid barrier defects in dogs with AD although there is still insufficient evidence for the benefit of lipid-containing topical formulations to recommend these as monotherapy for canine AD. The benefit of topical EFA-containing formulations is likely minimal in dogs already fed EFA-rich diets or EFA supplements. There are no studies evaluating the role of topical lipid formulations in cats.

C. Reduction of pruritus and skin lesions with pharmacological agents

i.) Topical glucocorticoids: although topical therapy is used less frequently in allergic cats than in dogs due to their fastidious grooming behaviour, the availability of rapidly absorbed products makes this option appealing. 0.0584% hydrocortisone aceponate spray is effective for the management of feline allergic skin disease. Care must be taken with frequent application as cutaneous atrophy has been reported with repeated use. Although the product is currently not licensed for use in cats, it appeared to be well tolerated, safe, and effective.

ii.) Oral glucocorticoids (prednisolone or methylprednisolone) and ciclosporin: these drugs are effective for treatment of chronic FAS, concurrently with or after control of known flare factors. Glucocorticoids lead to faster improvement than ciclosporin, but ciclosporin can be combined with oral prednisolone for the first three weeks to speed its onset of clinical improvement.

Oral glucocorticoids (prednisolone or methylprednisolone) should be used at 1 mg/kg once to twice daily to induce remission of clinical signs of FAS. After such remission occurs, the dose of oral glucocorticoids should be tapered to the lowest dosage and frequency that maintains an absence of signs to minimize the risk of side effects in the long term. Long acting injectable glucocorticoids should be avoided wherever possible as the lack of ability to taper their dose increases the risk of adverse events.

Oral ciclosporin (Atopica® for Cats) should be administered at 7mg/kg once daily with food until satisfactory control of clinical signs, which will usually take 4 to 6 weeks. Higher dosages of 10 to 15mg/kg for up to 12 weeks can be required for some eosinophilic dermatoses, particularly oral eosinophilic granuloma. Thereafter, the dose required to maintain remission should be tapered by decreasing the frequency of treatment from every day to every other day and then twice a week. Approximately 50% of cats can be maintained on ciclosporin twice a week without deterioration of the clinical signs. The frequency and the extent of dose tapering is more effective in cats that in dogs.
Adverse reactions in cats receiving ciclosporin include gastrointestinal signs (vomiting, diarrhoea and reduced appetite) which are usually mild and transient. Hepatic lipidosis can occur secondary to persistent weight loss. Strategies to reduce the frequency and severity of gastrointestinal signs include freezing the capsule (if used in lieu of liquid) prior to administration or dividing the liquid dose and giving it twice daily, or beginning at a lower dosage and gradually increasing to a therapeutic dosage.

Gingival hyperplasia has been reported in cats receiving ciclosporin. Drug cessation typically results in improvement. Oral azithromycin and azithromycin toothpaste are of benefit to dogs but have not been used in cats. Plaque control has shown to be of benefit in cats.

An important potential adverse effect of using ciclosporin in cats is the increased risk of developing systemic toxoplasmosis. Fatal toxoplasmosis in cats receiving ciclosporin occurs by reactivation of a latent infection or primary exposure in naïve cats. The risk for fatal toxoplasmosis is higher in cats with trough CsA concentrations >1000ng/ml and in cats receiving concurrent prednisolone administration. Routine measurement of ciclosporin trough concentrations (20 hours after dosing) in cats 1 to 2 weeks following commencement of therapy to detect at risk cats is mandatory. Further recommendations to reduce the risk of this disease include feeding only processed foods (or if meat is fed then cooked or frozen/thawed), avoiding raw meat, poultry, viscera or bones and preventing hunting and scavenging. When seroconversion occurs, or significant rises in toxoplasma antibody titres are observed in association with developing clinical illness in cats which were seropositive prior to initiation of immunosuppressive treatment, anti-toxoplasma chemotherapy should be commenced immediately to prevent acute systemic disease.

Ciclosporin administration is not recommended for cats that have a positive FIV or FeLV viral status or a history of neoplasia. Ciclosporin may precipitate relapse of feline herpesvirus infection, but these signs are usually mild and self-limiting. The risk of significant herpes relapse may be greater in cats with trough CsA concentrations > 1000ng/ml.

The long-term concurrent administration of oral ciclosporin and glucocorticoids (especially at higher dosages of either or both drugs) is not recommended because of the theoretical higher risk of immunosuppression predisposing to potentially severe opportunistic infections of the skin or other organs. There is no consensus on the need for laboratory monitoring (e.g. haematology, serum biochemistry and urinalysis) during prolonged ciclosporin or prednisolone administration; however, it is our recommendation that laboratory monitoring is performed on an annual basis. Due to the increased risk of urinary tract infections in dogs treated with oral glucocorticoids and ciclosporin in the long term, it is our recommendation that cats receiving these drugs should be monitored with annual urinalyses and urine cultures.
The concurrent use of allergen-specific immunotherapy, EFAs supplements or enriched diets might allow for a further reduction in the dose and/or frequency of oral glucocorticoids and ciclosporin required to maintain remission of clinical signs of AD. The efficacy and safety of these combined approaches has not yet been published.

iii.) Antihistamines: there is limited evidence of proven bioavailability and/or demonstrated reliable efficacy of antihistamines in cats and recommendations are anecdotal and based on open trials; success has been reported anywhere from 20 to 73%. A single, randomised, double-blinded, placebo-controlled, cross over study evaluated cetirizine administration at 1mg/kg q 24hrs in cats with mild to moderate cutaneous allergy and no improvement was noted in the population at the dose evaluated.

For optimal efficacy, this class of drugs is best used as part of a preventative strategy before a flare occurs; not during or after it; and they should preferably be administered on a continuous basis. The relatively mild and infrequent side effects make antihistamines a reasonable option, especially in patients with mild allergic disease. The author’s preferred antihistamines for cats include cetirizine 2mg/kg q 12 to 24hrs and hydroxyzine 1 to 2mg/kg q 12hrs.

iv.) Tricyclic antidepressants (TCA) and selective serotonin reuptake inhibitors (SSRI): in human patients with AD, psychological factors can contribute to the severity of clinical signs. Insufficient evidence exists regarding the role of these factors in cats with FAS but it is probable that environmental or social stress contributes to the pruritic threshold and the exacerbation of clinical signs in some patients. In cats that are not responding to conventional therapy for FAS, evaluate for psychogenic factors and consider using a fluoxetine 0.5 to 1mg/kg q 24hrs, or paroxetine 0.5 to 1mg/kg q 24hrs or sertraline 0.5 to 1mg/kg q 24hrs. Contraindications include hepatic, renal or cardiac disease and drugs must be administered for 4 to 6 weeks before evaluating clinical response.

v.) Biotherapeutic immunomodulators: recombinant interferons: recombinant canine interferon-gamma (Interdog ®), given subcutaneously at 5,000–10,000 units/kg three times weekly for 4 weeks, then once weekly, is effective for treatment of canine AD. Recombinant feline interferon-omega (Virbagen omega ®), administered subcutaneously or orally, has been shown to provide some inconsistent reduction of skin lesions and pruritus in dogs with AD. There are no studies published using feline interferon-omega in cats with FAS.

3. Implement strategies to prevent recurrence of signs
   A. Implement proactive topical pharmacotherapy: in humans with AD, there is evidence for the high benefit, cost effectiveness and low risk of proactive intermittent applications of topical glucocorticoids and tacrolimus to previously affected skin areas to delay or prevent the appearance of such flares. There is currently no evidence for the effectiveness of a similar approach in cats with FAS, but the possible benefit, low risk and low cost suggest that such strategy is worth considering in suitable cases. In dogs, the application of a topical hydrocortisone aceponate spray (Cortavance ®) to areas of previous skin lesions, two consecutive days each week, can delay the recurrence of lesions at these sites without causing visible skin atrophy. A similar strategy could prove useful in cats.
B. *Implement allergen-specific immunotherapy:* no controlled studies have been performed to determine the value of allergen-specific immunotherapy (ASIT) as a modifying treatment for cats with FAS; much of what has been reported in cats is based on open trials and anecdotal information. Despite this, ASIT is considered to be a safe and effective treatment option for FAS in cats. Reported success rates in uncontrolled studies vary from 50% to 100%. As in dogs, no single or standardised immunotherapy administration protocol exists for cats with FAS. Interval between injections, injection volume, and allergen concentration are all highly variable between dermatologists; the schedule of administration is typically extrapolated from the preferred canine protocol.

Conventional injection ASIT typically includes an induction phase, where gradually increasing amounts of allergen are administered over a period of several weeks and a maintenance phase, where injections are typically administered every 1 to 3 weeks. Side effects are rare and may include localised pruritus or anaphylaxis.

Rush immunotherapy (RIT) is a technique of administering increasing amounts of allergen in a hospital or clinic setting with careful monitoring over several hours until the maintenance dose is reached. This option for immunotherapy administration has been evaluated in dogs and humans, with few adverse reactions reported. A pilot study in a small number of cats suggested that rush immunotherapy was safe and effective. It is uncertain whether improved efficacy is noted with this method as compared to conventional administration protocols, however, the benefits of rush immunotherapy are that it condenses the induction phase into only a few hours. Our dermatology clinic performs all immunotherapy protocols in dogs and cats using RIT and the reduced burden of frequent injections at the beginning of ASIT and improved owner compliance are definite advantages of this approach.

There is some evidence that ASIT administered via the sublingual route (sublingual immunotherapy; SLIT), are safe and effective for treatment of atopic dogs, but there is no published information evaluating sublingual immunotherapy in cats with FAS. Anecdotally, benefits have been observed for some cats where SLIT was used as part of the therapeutic protocol. It appears to be well tolerated by most cats. The formulation is palatable and even fastidious cats seem to tolerate the small volume necessary for administration. Studies are needed to evaluate the relative efficacy and safety profile in a large number of allergic cats to compare sublingual versus conventional immunotherapy. SLIT can be a useful alternative for cats and owners adverse to the administration of subcutaneous injections.

Most cats that demonstrate a response to ASIT exhibit a good response within 6 to 12 months. Because the onset of clinical benefit might not appear for months, ASIT must be continued for at least one year to properly evaluate its efficacy. For most allergic cats, concurrent medication is necessary especially during the induction and early maintenance phase of immunotherapy administration. There is currently no evidence suggesting that the concurrent administration of such drugs alters the clinical benefit of ASIT. Efficacy can still be assessed based on the ability to lower concurrent medication doses and potentially discontinue certain medications in favour of safer options.
Whether or not ASIT must be continued for the reminder of the life of cats with FAS has not been established. While most patients appear to require many years of ASIT, attempts should be made to decrease the frequency of administration, or even stop this intervention, in cats exhibiting a prolonged complete remission of signs.

Conclusion
In summary, the treatment of FAS must be an individual prescription for each patient and in the majority of cases, a combination approach is required. Treatment can be challenging and should incorporate identification and elimination of flare factors, a reduction of skin lesions and pruritus, protection of the skin barrier and prevention of recurrence of signs after remission. Not all treatments will be suitable for every patient and not all drugs will be equally effective for, or tolerated by, every cat. We should try to abide by evidence-based veterinary principles and at the same time consider the cost and ease of the various treatment options and the quality of life of each patient.

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STRESS AND THE ALLERGIC CAT BEYOND DERMATOLOGY — HOW TO REDUCE STRESS BOTH IN THE CLINIC AND THE HOME

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Introduction
Minimising the stress of a hospital visit or stay for a cat requires a good understanding of feline behaviour along with empathy for the patient and its experience. Knowledge of normal cat behaviour is essential to be able to predict and understand stressors for the cat. Prevention of stress in the clinic makes visits more successful for both the veterinarian and owner; for example, vital parameters are more reliable and physical examinations can be completed. In turn, a pleasant and positive experience means that the client is more likely to return to the clinic for follow-up or when new problems develop, building a long-term partnership of care between the owner and clinic. Recognition of stress in the home environment is important too; owners may not readily identify possible stressors and these could potentially impact a wide-range of conditions from inflammatory diseases (e.g., IBD, idiopathic cystitis) to inappropriate urination and psychogenic alopecia.

The stressed cat
The stress of visiting the veterinary clinic may induce anxiety and fear, which can precipitate a variety of behavioural responses. Aggressive behaviour is commonly misunderstood to be an inherent temperament ‘issue’ with cats being described as wild, vicious, aggressive, grumpy or nasty in clinical records. The recognition of fear and fear aggression as potential responses to stress is essential in developing a good cat friendly approach. The terms ‘fearful’ and ‘fear-aggressive’ are preferred and show both understanding and empathy.

With this in mind it is easy to recognise stress in the fearful cat that is behaving aggressively. However, some cats will manifest stress or fear much more subtly such as simply withdrawing, sitting at the back of the hospital cage under bedding, refusing to eat or by trying to minimise their interactions with staff. There is a scale of behavioural signs that cats may exhibit in the clinic, with the ‘worst’ response being human directed fear aggression, which typically reflects a marked fear or stress response by the cat. This is an extremely negative scenario for the cat and owner, as well as hospital staff, and can usually be avoided by using a cat friendly approach. Assessment of ear position, body posture and tail movement can be used as indicators of a cat’s state of arousal.

Scale of stress behaviour responses:
- Reluctance to come out of the carrier
- Sweating paws, avoidance of any eye contact
- Tail swishing
- Vocalisation
- Ears held flattened to head, piloerection, mydriasis
- Vocalisation, hissing and spitting
• Urination, defaecation on handling
• Ears held erect but turned back, low growl
• Swiping and biting

For a domestic pet cat to reach the stage of exhibiting aggression means earlier fear cues may have been missed or could reflect prior negative experiences. From the cat’s perspective a ‘fright and flee response’ is not possible (preferred choice), leaving the cat with the only option of ‘fright and fight’.

1. The normal cat and its behaviour
In order to consider sources of stress in the clinic (and at home), a few key facts need to be understood regarding the cat’s normal behaviour
A. The cat is typically a solitary creature, choosing to live in a colony setting only where food sources are plentiful. Cats may choose to form social groups in multicat households.
B. Cats have a home range, the area over which they will roam and a territory, an area of the home range that they will scent mark and be prepared to defend.
C. Cats are obligate carnivores and naturally would hunt frequently through the day, eating multiple (10-12) small meals. Hunting activity is greatest at dusk and dawn.
D. Cats prefer to toilet away from water and food sources; they prefer to drink away from the site of hunting.
E. The cat’s sense of smell is highly developed; scent marking is utilised to communicate information regarding territory to other cats.
F. ‘Fright and flee’ is a preferred response to ‘fright and fight’

2. Reducing stress in the clinic – developing a cat friendly approach
To reduce stress for the cat visiting the clinic it is helpful to try to see the situation through the cat’s eyes and imagine all the potential novel sights, sounds, smells and contact that the cat may encounter. Positive early experiences are essential in preventing the cat from developing a negative association with visiting the clinic. The entire veterinary team needs to be briefed in understanding the specific requirements of cats, to make sure that a cat friendly approach is practised and conveyed to the client, from the point of a client making an appointment all the way through to discharge after a hospital stay.
A. Travelling to the clinic
   i.) Discuss carrier choice, or provide information on your practise website; the most useful types are top loading carriers and those that the lid of the carrier can be removed to allow the cat to be examined within.
   ii.) Encourage the owner to acclimatise the cat to the carrier; leave the carrier out at home for a few days before the visit (or long-term so that the cat perceives it as part of the furniture or a resting place).
   iii.) Place familiar used blankets/towels inside the carrier, spray with Feliway™ at least thirty minutes before travelling.
   iv.) Advise the owner to travel the cat fasted, to reduce the risk of motion nausea and ensure the carrier is secured in a seat by a seat belt.
   v.) Advise the owner to cover the carrier en route with a towel or blanket and keep noise within the car to a minimum (avoid loud radio sounds).
B. The waiting room
   a. Ideally cats should wait in a separate waiting area from other animals, however if
      this is not possible consider the following:
      (a) Whether an area of the waiting room can be screened off or physically
          separated for cats and their owners? Owners of dogs should be asked not to
          use this area of the waiting room.
      (b) Whether there is a spare consulting room that can temporarily be used as a
          waiting area for the cats and their owners?
      (c) Whether you can operate separate consulting times for cat and dog clinics?
      (d) Would owners with fearful cats be prepared to wait in their car until their
          appointment is called? Be flexible in being prepared to spend more time with
          fearful patients (e.g., book a double consultation slot or book at the beginning
          or end of a consultation) block, when you will be less pushed for time.
   b. Provide space for cat carriers to be placed off of the ground such as chairs,
      shelves, side tables.
   c. Provide clean blankets or sheets for owners to place over carriers.
C. The consulting room and examination
   a. Allow the cat time to acclimatise if possible — open the carrier and allow the cat
      to come out of its own accord. Assess whether the cat looks anxious or relaxed. If
      the cat is not willing to come out, remove the lid if the carrier is top loading. For
      front loading carriers, gently reach in and support the caudal abdomen and back
      legs to encourage the cat to move forward; avoid scruffing the cat to pull it out or
      tipping the carrier end up to shake the cat out.
   b. Be flexible in how you examine the cat; most cats will settle on a consulting room
      table that has a clean soft bed laid on it, while others prefer to be examined on the
      floor, in the base of their carrier or on the owner’s lap. If the room has a low
      windowsill this is often an area the cat will prefer to sit on and be distracted by
      external things.
   c. Handle the cat gently and slowly and remember to speak in a quiet voice; make
      small and slow movements around the cat, examining the cat with its head facing
      away from you initially; staring at the cat may be perceived as confrontational.
      Minimal restraint is preferred; scruffing is not permitted in the author’s clinic.
   d. Avoid ‘shussing’ sounds, which may sound like a ‘hiss’ to a cat.
   e. If the cat is starting to get fidgety, or you notice early fear/stress signs (tail
      swishing, vocalisation), stop and allow a break before trying to complete the
      examination.
   f. Leave the most aversive parts of the examination until last such as taking the cat’s
      temperature or opening the mouth fully.
   g. Be mindful that some cats may be averse to certain parts of the examination (e.g.,
      handling around the head if chronic aural disease or hypertensive). Osteoarthritis
      is a common reason for a cat to resent parts of the examination or positioning for
      venepuncture.
   h. Large towels are very useful for handling fearful cats; the majority of the cat can
      be secured gently within the towel and sections lifted to examine different areas of
      the body; providing somewhere to hide enables the cat to maintain some control.
      Gauntlets, cat bags and cat muzzles are not used in the author’s clinic; whilst
some find these useful, our experience is that these generally are ineffective and more stressful for the cat. Sleeves covering the entire arm provide some protection for personnel (where usually there is a ‘bare below the elbow’ policy).

i. Consider whether sedation would be in the best interest of the patient and staff for very fearful and fear-aggressive patients (high dose butorphanol can be helpful for mild fear or stress 0.3-0.4mg.kg IM)

j. Have Feliway™ diffusers in the consultation room.

k. Use treats to develop a positive association with examination; wand toys can also be a useful distraction, particularly for kittens.

l. Minimise strong smells in the consult room from perfumes, air fresheners or disinfectants.

m. Be prepared to involve the owner if appropriate (remember that the owner’s anxiety may be sensed by the cat and have a negative effect); ask owners to avoid rough handling or reprimanding the cat.

D. The hospital ward

i.) Ideally cats should be hospitalised in a separate ward from dogs where possible, or at least out of visual contact from other dogs (and cats). The ward should ideally not be a thoroughfare.

ii.) Provide a resting/hiding space within the cage (cardboard box, feline fort, cat’s own carrier if space allows); line the bottom of the cage with soft bedding or a soft mat.

iii.) Consider retaining one of the client’s own blankets or towels to use in the hospital cage to provide a familiar scent.

iv.) Place the litter tray as far away from the food and water bowls as possible.

v.) Spot-clean and remove dirty bedding each day rather than removing all of the bedding and disinfecting entirely, to enable the cat to develop a familiar scent in the cage.

vi.) Be aware of noise levels within the ward and try to keep to a minimum. Avoid rounds type discussions in front of the cage.

vii.) Avoid doing procedures in front of cats within cages (e.g., placing IV catheters or taking blood); this has been shown to raise stress levels in susceptible cats.

viii.) Cover the front of the cage of very fearful cats when observations are not being taken.

ix.) When moving cats around the hospital cover the cage with a blanket or towel.

x.) Use wide and shallow food and water bowls; discuss food preferences with clients on admission and try to match the food offered in the hospital to the home ration. Making a change to a veterinary therapeutic food is not recommended in the hospital environment due to the risk of developing a food aversion.

xi.) Low-level lighting is preferable (or at least periods when the light level is reduced) to promote rest and sleep.

xii.) Feliway™ diffusers should be active in the ward, and consider the application of Feliway™ spray to bedding before placing a cat into the cage for the first time.

E. Procedure tips

a. Think about the order of procedures carefully for individual patients. It may be obvious to suggest doing the least aversive first; however, this is not always remembered when a hospital is busy.
b. Aim to do all procedures in conscious unsedated cats in a quiet room, away from other patients (this includes daily examinations).
c. Use gentle restraint techniques, often ‘less is more’ and watch for signs of increasing stress, fear or arousal.
d. Local anaesthetic is useful for venepuncture and intravenous catheter placement (EMLA applied to clipped skin 30-45 minutes in advance); use 23G needles and 22G or 24G IV catheters (Jelco are well tolerated).
e. Use quiet coat clippers (Braun Aesculap ISIS clippers).
f. Use paediatric scales for weighing cats, having these in consultation and procedures rooms, reduces movement of cats around the hospital.
g. Use soft buster collars and minimise dressings that inhibit the cat’s movement.
h. Consider using sedation or anaesthesia in very fearful or fear-aggressive patients; crush cages are not recommended for domestic pets for administering drugs since this will only heighten a negative experience and will likely make the cat even less manageable at the next visit.

F. Returning home
a. Involve the client in medication decisions if on-going treatment is required (e.g., are tablets or liquid formulas better tolerated by the cat).
b. Arrange a discharge appointment with a member of the team who will be competent to discuss and demonstrate methods of administering medications or nursing techniques that may be required.
c. Provide educational resources for the owner to refer to at home such as links to videos demonstrating medication administration, detailed written instructions regarding the medication, administration and possible side effects.
d. For cats from a multi-cat household there may be negative reactions from other cats towards the ‘patient’ cat, since it may now smell of the hospital environment. Prepare the owner for how to manage the reintroduction of the cat. For example, transferring scents between the two cats can help minimise the response by the cats at home to the ‘patient’ cat. Further details are provided in the AAFP and ISFM Feline-friendly Handling Guidelines.

3. Reducing stress at home – understanding group interactions and resource management
   When considering whether a cat is stressed in the home environment, an initial assessment can be made by evaluating whether the individual cat’s environmental needs are met and how it interacts with other household members (pets and humans).
   A. Core resources or ‘five pillars’ of a healthy feline environment (from the AAFP/ISFM guidelines on feline environmental needs)
      i.) Provision of a safe place to rest and/or retreat to
      ii.) Provision of multiple and separated key environmental resources
         a. Food
         b. Water
         c. Toileting area
         d. Scratching area
         e. Play area
         f. Resting or sleeping area
      iii.) Opportunity for play and predatory behaviour
iv.) Positive, consistent and predictable human-cat social interaction
v.) Provision of an environment that respects the importance of the cat’s sense of smell. Assessing each of these factors individually will enable the veterinarian to identify the provisions that require increased access or modification, to help the owner to optimise the environment for the cat and reduce possible stressors.

B. Interactions with other pets
A thorough understanding of how the patient cat interacts with other pets in the household must be developed. Inter-cat conflict is an important potential stressor. Owners may misunderstand certain behaviours. For example, aggression may be perceived to be play fighting. A starting point is to establish the feline social groupings within the household; cats that allogroom, allorub (body rubbing and tail wrapping), rest or sleep in contact or very close proximity and play together are considered to belong to the same social group. Building up a picture of the patient cat’s position within the household groups may help to identify inter-cat conflict.

For further detail please refer to the AAFP and ISFM Guidelines on Feline Environmental Needs. The value of involving a qualified veterinary behaviourist in managing feline stress and anxiety related medical conditions cannot be emphasised enough, consultation or referral to clinicians experienced in this area should be considered.

Selected References
   Video clips providing detailed information on how to make your practice feline-friendly.
   Information on developing a cat friendly clinic (Scheme outside of the USA)
   AAFP website pages on the American Cat Friendly Practice program.
8. www.youtube.com/user/iCatCare. Useful set of owner-directed videos to instruct administration of medications by various routes; highly recommended.
Introduction and principals for allergen specific immunotherapy

Allergic skin disease in cats and dogs are managed similar despite differing opinions with regards to the pathogenesis of canine atopic dermatitis and feline cutaneous allergy. Therapeutic options consist of symptomatic medical treatment and causative allergen specific immunotherapy (ASIT). Often various treatment options are combined to provide the maximum clinical benefit and minimize the amount and severity of adverse effects.

ASIT also known as hyposensitization or desensitization is a common treatment for atopic disease in humans, dogs, cats and horses. The WHO defines ASIT as the ‘incremental administration of increasing quantities of an allergen extract to an allergic patient’. The goal of successful therapy is to reduce or eliminate the clinical signs associated with repeated exposure to the causative allergens. Conventional ASIT consists of a subcutaneous injection protocol with an induction and a maintenance course. The induction course consists of subcutaneous injections of increasing doses of allergen extract until the maintenance dose (usually 20,000 PNU) is reached. Maintenance therapy is injected at intervals of 1-4 week (dependent on the allergen source used) usually for several years. It is commonly recommended to continue ASIT for at least one year to see full effect.

Allergens for ASIT are chosen based on intradermal test results and/or in-vitro serology for allergen specific IgE as well as clinical symptoms. In humans, there is good evidence that ASIT can significantly reduce symptoms of seasonal allergic rhinitis and allergic asthma with a low risk of severe adverse effects. In contrast, only a few studies exist evaluating ASIT for atopic dermatitis in humans and it is unclear whether ASIT improves the condition.

In veterinary medicine, double-blinded randomized studies evaluating immunotherapy are scarce. Most studies are retrospective and cannot be compared easily due to different injection protocols, allergen types, sources and preparations (in Europe, most allergens are Alum or Calcium phosphate precipitated, whereas in other parts of the world these are mainly aqueous). Nevertheless, the outcome of most of these studies is similar accounting for an overall clinical success rate of 50-100%.

Mechanisms of action

The mechanisms of action of allergen specific immunotherapy are not completely elucidated. Most of the data originates from human bee and wasp venom allergy. Briefly, allergen-specific IgG concentrations, particularly IgG4, rise during ASIT blocking the IgE-mediated reactions by binding to the same epitopes as the allergen specific IgE. Regulatory T-cells (Treg) are activated and increase during ASIT producing TGF-β and IL-10 thus limiting the inflammatory response.
by down-regulating the inflammatory processes. IL-10 levels correlate with clinical efficacy of ASIT.

In dogs a significant increase in IgG4 concentrations were noted within 6 months of therapy and a decrease in allergen specific IgE after 12 months. A more recent study observed a significant increase of Treg cells and IL-10 within 3 months of ASIT, which correlated with the clinical response rate. In cats, no such studies have been done.

1. Allergen specific immunotherapy for cutaneous allergies

Over the last 40 years, ASIT has become a focus of interest also in veterinary dermatology and is now widely used in the veterinary community as the only specific treatment for canine atopic dermatitis (CAD). Numerous studies suggest that ASIT is a safe and efficacious treatment for CAD with success rates ranging from 45 to 100% dependent on factors like allergy testing methodology, allergen type and source, induction and maintenance protocols, dose and concentration of allergen as well as response criteria. However, scientific information with a high level of evidence for or against the efficacy of ASIT is scarce and the literature only contains few prospective and blinded studies. Scientific information regarding ASIT in feline allergic disease is rare and mostly anecdotal. However, some evidence exists that ASIT should be considered a treatment option for allergic disease in cats. As in the canine patient, no standardized protocols for ASIT in cat with cutaneous allergies exist. Intervals between injections, volumes and allergen concentrations are highly variable between laboratories providing ASIT for the feline patient.

A. Subcutaneous immunotherapy (SCIT) for feline allergic dermatitis

Compared to the human or canine literature only a handful reports exist regarding the efficacy of allergen specific immunotherapy in allergic cats. An early survey evaluated the response of SCIT in 81 cats. Allergens were chosen based on IgE measurement by radioallergosorbent test. Overall response rate was 75.3% of cats showing an improvement of at least 50%. Interestingly, different allergic clinical presentations showed different response rates: 93% of the cats with eosinophilic granuloma, 60% of cats with self-induced alopecia and 62% of cats with allergic otitis responded. Only 10% of the cats did not respond to immunotherapy.

In open trial with 29 cats on aqueous ASIT following intradermal testing showed 6 with good to excellent response, 5 with moderate response. 3 cats had a poor response and nine were lost to follow up.

Ravens et al reported the outcome of 23 cats on at least 12 months immunotherapy. A ‘good’ response was observed in 57% ‘partial’ response in 26% and ‘poor’ response in 17% cats. In another publication two of four cats with cutaneous hypersensitivities (miliary dermatitis, pruritus, self-induced alopecia and eosinophilic granuloma complex) responded excellent to SCIT with allergens selected based on intradermal testing. Other studies have reported >75% improvement in 67% and 69% of cats respectively.

i. Rush Immunotherapy

Rush immunotherapy (RIT) has been evaluated in dogs and people as a safe alternative to conventional subcutaneous immunotherapy. Compared to conventional SCIT, the induction phase is reduced by injecting increasing doses of allergen extract
in short intervals. Maintenance dose is therefore reached within one day compared to several months with conventional SCIT. A double-blinded study comparing SCIT and RIT with aqueous allergens in dogs resulted in a shorter period until maximal improvement with RIT compared to SCIT.

Preferably RIT should be applied in a clinical setting where careful monitoring of vital parameters and side effects during the administration of allergen injections is possible at all times. Pretreatment with an antihistamine decreases the number and severity of side effects. Potential side effects are similar to those with ASIT: increased pruritus after administration, urticaria and anaphylaxis. Aqueous and alum-precipitated allergens have been used in RIT protocols in dogs. A RIT protocol with alum-precipitated allergens in 20 dogs with atopic dermatitis showed significant improvement in pruritus, clinical and medication scores after 12 months of therapy. The observed clinical response was good to excellent in 70% of the dogs with only one dog showing a side effect of RIT (vomiting during the induction day) suggesting that RIT with alum precipitated allergens in canine atopic dermatitis is safe and efficacious.

In general, RIT is well established in dogs showing a faster initial response and a marginally though not statistically significant better success rate compared to conventional SCIT.

Only one study describes a RIT protocol in a small number of cats: Allergens for ASIT were chosen based on a liquid phase immunoenzymatic testing in four cats. A five-hour rush immunotherapy protocol was conducted which was well tolerated, however, efficacy was not reported.

The main advantage of RIT lies in the abbreviated induction period thus avoiding confusion of owner and lengthy induction time hence resulting in better client compliance.

ii. Pullulan-conjugated SCIT
In humans pullulan-conjugated SCIT has been successfully used in people with Cedar pollen rhinitis. Pullulan, a polymer of \(\alpha\)-glucose, is used as an adjuvant to enhance the effect of immunotherapy.

A Japanese group developed a recombinant house dust mite allergen Der f 2, which was conjugated to pullulan to reduce IgE-binding activity and increase IgG production (“Allermune HDM”, Nippon Zenyaku Kogyo, Fukushima, Japan). In 143 dogs with atopic dermatitis due to house dust mite sensitization, Der f 2 pullulan was injected subcutaneously once weekly for 4, 5 and 6 injections. Lesion score (CADESI) significantly improved in all treated groups, whereas pruritus only improved in dogs receiving 5 or 6 injections. Serum Der f 2-specific IgG significantly increased in groups given five or six injections. Remission was maintained in 70% of dogs given 6 injections with no additional medication nor lesions or pruritus for at least 12 months after the final injection. Pullulan-conjugated SCIT appears to offer a new, safe and effective allergen immunotherapy for canine atopic dermatitis with a
long-term effect.

In cats no information of pullulan-conjugated SCIT is available.

In summary conventional SCIT and likely also rush immunotherapy following intradermal testing or in-vitro serology appear to be a valuable treatment option for cats with various clinical presentations of cutaneous hypersensitivities. Double blinded placebo controlled studies are needed to further elucidate the efficacy of SCIT in cats with manifestation of cutaneous allergies.

B. Sublingual immunotherapy (SLIT)

Sublingual immunotherapy is a more recent form of allergen administration in the formulation of drops. It has been a promising treatment modality in people mainly with allergic respiratory disease with considerably less side effects compared to SCIT.

In pets, the allergen extracts are applied twice daily to the oral mucosa. Especially for owners with a fear of needles or pets intolerant of injections, SLIT offers an appealing alternative. Only few studies exist in veterinary medicine documenting the efficacy of SLIT. A large open multicenter study enrolled 217 atopic dogs for sublingual immunotherapy. All dogs underwent twice daily oral immunotherapy based on individual allergy test results. After 6 months, 55% of dogs had a good-to-excellent response to SLIT. Dogs that had not received previous immunotherapy, a good to excellent response rate was observed in 59% of dogs. In dogs, which had undergone previous conventional SCIT, 49% had a good-excellent response to SLIT.

In another prospective open study in 10 dogs with spontaneous CAD due to dust mite sensitivity, SLIT was proven efficacious in 80% of the enrolled dogs. After 6 months owner’s assessment for improvement ranged from 0% (2/10) to 100% (2/10) with a median improvement of 72.5% in 80% of the dogs. Prospective outcome measures showed reduction of lesions, pruritus and a significantly decreased need of oral steroids (mean dose of methylprednisolone initially 10.2mg/kg, at the end of the study four dogs no longer needed steroids). This improvement was associated with a significant decrease in dust mite specific serum IgE.

In dogs, therapeutic efficacy of SLIT appears to be similar to that reported for conventional SCIT and might offer a safe and effective treatment for CAD, including dogs which had failed previous SCIT.

In cats, no published scientific data exists on the effect or safety of sublingual immunotherapy in allergic cats. Anecdotally, efficacy has been seen in some cats where SLIT was used to help control atopic dermatitis.

So far there is no scientific evidence for or against the use of SLIT in cats with cutaneous hypersensitivities however, anecdotal data support the use of SLIT in cats with cutaneous allergic manifestations. Studies are needed to evaluate the safety and efficacy of sublingual immunotherapy in cats with cutaneous allergic disease.
**Feline asthma**

Feline asthma is a common chronic lower airway inflammation associated with coughing, wheezing and expiratory distress due to an allergic cause. As in humans, feline asthma is believed to develop secondary to a type-1 hypersensitivity to aeroallergens. Evidence for an allergic reaction as a predominant cause for feline asthma is based on various experimental models, epidemiologic studies and observations of improvement by allergen avoidance strategies and response to ASIT.

Typical signs of feline asthma include eosinophilic airway inflammation, airflow limitation and chronic airway remodeling resulting in declining lung functions. Commonly, feline asthma is treated with topical and/or systemic corticosteroids, bronchodilators and inhalation therapy. As in cutaneous allergies, such therapeutic strategies only offer palliative suppression of inflammation, whereas ASIT induces immunologic tolerance to the offending allergen thus offering a potential for cure. Further, chronic corticosteroid use can result in unpleasant severe adverse effects. A causative therapy to reduce the allergic response would therefore be a desirable treatment in feline asthma. Allergy testing by intradermal testing or serum allergen-specific IgE can be used to identify the offending allergens, although these are not commonly used in cats with feline asthma. A reason for this neglect might be the fact that asthmatic cats are rarely referred to dermatologists but are seen by internists. Nevertheless, with appropriate identification, allergen avoidance and ASIT might be helpful to reduce or cure clinical symptoms in affected cats.

1. **ASIT for feline asthma**

   Allergen specific immunotherapy was studied in several - though mainly experimental studies - in cats with allergic asthma. In contrast to experimentally induced asthma where the triggering allergen(s) are well known, it is a major challenge to treat naturally occurring asthma where allergenic triggers may be seasonal or influenced by concurrent therapy.

   A. **Subcutaneous immunotherapy for feline asthma**

      Only few reports of naturally occurring feline asthma treated by SCIT exist. Halliwell describes 81 cats with various forms of allergic disease, which were treated with conventional SCIT based on a radioallergosorbent test to define the offending allergens. 86.1% of the cats with allergic asthma showed at least 50% improvement on SCIT. The results suggest that confirmation of a suspected diagnosis of allergic disease in cats by means of in vitro tests and subsequent therapy with hyposensitization should be a major consideration in feline practice.

      i. **Subcutaneous rush immunotherapy for feline asthma**

         RIT was evaluated in an experimental model of feline allergic asthma due to Bermuda grass sensitization. RIT involved increasing subcutaneous doses of Bermuda grass allergen over two days. Cats were followed over 6 months. In treated cats, a airway eosinophilia decreased concurrent with an induction of hyporesponsive lymphocytes and alterations of cytokine profiles (increased IFN-γ and IL-10).

      ii. **Intranasal rush immunotherapy in feline allergic asthma**

         One experimental study evaluated the effect and safety of an intranasal rush immunotherapy protocol in cats with allergic asthma due to experimentally induced Bermuda grass sensitization. Cats were randomized and allocated to subcutaneous or intranasal rush immunotherapy. Both groups showed decreased eosinophilic airway inflammation after RIT. Subcutaneous RIT demonstrated
more consistent resolution of clinical signs after allergen challenge but showed
more though less severe adverse events compared to intranasal RIT. Either
protocol could be considered for the treatment of feline allergic asthma with the
subcutaneous protocol appearing more effective.

 iii. Cytosine-phosphate-guanine (CpG) oligodeoxynucleotides (ODN) rush
immunotherapy in feline allergic asthma

CpG ODNs are DNA sequences that are common in microbes. In mammals
CpG ODNs induce a strongly polarized Th1 immune response, which has been
proposed as a mechanism to prevent the Th2 immune deviation characterizing
asthma. Data in animal models of asthma treated with CpGs show a reduction of
serum IgE production, airway hyperreactivity and airway eosinophilia.

In a crossover study comprising 12 cats with experimentally induced allergic
asthma to Bermuda grass allergen, the clinical benefit of CpG-ODN was studied.
Six cats were treated with adjuvanted RIT using CpG ODN over two days, and
six received placebo (saline) RIT. Over two months, CpG treated cats showed a
dampened eosinophilic airway inflammation and a reduction of IL-4 in
bronchoalveolar lavage fluid. The CpG-RIT further appeared to be safer
compared to a historical non-adjuvanted RIT protocol.

B. SLIT

No published studies exist evaluating sublingual immunotherapy in feline asthma.
However, an online survey of an international listserve showed that some dermatologists
use SLIT as a treatment for feline asthma with as good results as with SCIT.

2. Mesenchymal stem cell therapy for feline allergic asthma

Trzil and co-authors evaluated the effect of mesenchymal stem cells (MSCs) in nine cats with
experimentally induced chronic allergic asthma to Bermuda grass pollens. Cats were either
treated with six intravenous infusions of MSCs or placebo bimonthly and were evaluated
over one year. MSC treated cats showed less airway remodeling than the placebo group, but
there were no differences between groups in respect to airway eosinophilia
hyperresponsiveness.

A second study by the same authors involved six cats with experimentally induced acute
allergic asthma to Bermuda grass allergen, which either received five intravenous infusions
of adipose-derived MSCs in various concentrations or saline as a placebo (n=2) over the first
130 days of asthma induction. Outcome measures nine months after treatment included
airway eosinophil percentage airway eosinophilia, pulmonary mechanics, thoracic computed
tomography and several immunologic assays. Improvement was significant for all parameters
after approximately five months. After 9 months, MSC-treated cats showed normal
eosinophil percentages and decreased airway remodeling. MSCs may have a delayed effect in
reducing airway inflammation airway hyperresponsiveness and remodeling in experimentally
induced feline allergic asthma.
Results of an online survey regarding the use of allergen specific immunotherapy in cats.
An online survey was performed by sending out a questionnaire to an international veterinary dermatology list-serve (Table 1). Twenty-nine veterinarians sent back the questionnaire (24 specialists, 5 general practitioners of whom four have a sub-specialization in dermatology) of whom more than 86% (25/29) use SCIT for cutaneous allergies and 58% (14/24) also treat allergic airway disease with SCIT. 25% (6/24) responders also apply sublingual immunotherapy in cats and two responders perform RIT. The selection of allergens for ASIT is equally chosen either based on intradermal testing, serology or a combination of the two. The majority of responders (72%) use aqueous allergens, 20% use alum precipitated and only 8% use Calcium phosphate bound allergens. Depending on the allergen type, injection protocols range from once weekly to once monthly and are adjusted in dosage volume and interval, as needed based on individual patient response.

In 76% of the cats treated with SCIT, owners inject the allergy vaccines at home whereas 24% bring their cat to the veterinarian. The success rate for ASIT was generally rated to be good to very good: Eighty-four percent (21/25) responders estimate the success rate for SCIT for an overall improvement of more than 50% to be at least 50%-75%. And 36% (9/25) recorded that >75% of the cats improve > 50%. This success rates were independent from the allergic disease treated.

Of the 25% responders which use SLIT in addition, 5/6 estimated their success rate for >50% overall improvement to be more than >75%. One person recorded 50-75% success rate but only applies SLIT once SCIT failed.

Severe side effects were merely recorded in the group with SCIT. Four responders have seen anaphylaxis due to SCIT. Other side effects recorded were local reactions (pain at injection site, swelling, erythema), increased pruritus and vomiting. In the SLIT group, the most common side effect was vomiting; other side effects included increased pruritus and pawing at the mouth.

In conclusion, among veterinary dermatologists SCIT is widely used as a treatment modality for cutaneous allergies as well as allergic airway disease. The efficacy seems to be equally high as in canine atopic dermatitis. Side effects are rare but can be severe. Even though SLIT is not used as commonly in feline allergic patients, it appears to be effective and safe. Cats seem to tolerate oral immunotherapy well even though twice-daily oral application can be a challenge in cats.

Summary
In summary, there is ample evidence that ASIT in dogs is effective, but as most studies were not blinded, outcome measures often subjective, statistical evaluation frequently suboptimal and the power of the studies generally low, there is a need to look at ASIT in animals with well-designed, long-term, double-blinded studies. When it comes to therapeutic effect and safety of ASIT in cats, information is even sparser and further studies are urgently needed. Based on an online survey, SCIT is routinely used among dermatologists as a treatment modality for feline allergic asthma and manifestations of cutaneous allergies with a subjective overall improvement of >50% in 50-75% of the cases. Some dermatologist also use SLIT to treat the
above diseases with the same therapeutic effect but less side effects. However, cats tend to
tolerate injections better than oral application of the immunotherapy.

Studies are needed to elucidate the effect and safety of allergen specific immunotherapy in cats.

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Vogelnest, Lisa Graziano, Valerie Fadok, Kathy Morris, Rendina McFadden, Mona Boord, Pat
White, Nicole Heinrich, Annette Peterson, Beth McDonald, Katrin Timm
Table 1: Feline immunotherapy – results of an online survey

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<tr>
<th>Category</th>
<th>No of responders</th>
<th>Comments</th>
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</thead>
<tbody>
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<td></td>
</tr>
<tr>
<td>GP with specialization in dermatology</td>
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<td></td>
</tr>
<tr>
<td>Derm resident/diplomate</td>
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<td>Percentage of feline patients</td>
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<td>41-50%:</td>
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<td>Uses ASIT in allergic cats</td>
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<td>Yes:</td>
<td>25</td>
<td>Specialist: 2 ; GPderm: 1, GP: 1</td>
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<td>Allergy tests used</td>
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References


GENETIC SKIN DISEASES IN CATS
Tosso Leeb

Institute of Genetics, University of Bern, Bern, Switzerland
DermFocus, University of Bern, Switzerland

Introduction
Compared to dogs and horses, only relatively few genodermatoses are known in cats. It is not fully clear whether cats truly have less genetic skin problems or whether genetic skin problems in cats just have not been recognized to the same extent as in dogs and horses. In the following chapters I will try to summarize the available knowledge on genodermatoses in cats.

Genodermatoses with Known Genetic Cause
In this group of traits, genetic testing is available. This is mostly relevant for breeding purposes in order to recognize non-affected carriers.

Hairlessness with short life expectancy (OMIA 001949-9685)
Marie Abitbol and her French colleagues solved the genetics of this lethal defect in Birman cats (Abitbol et al. 2015). The mode of inheritance is monogenic autosomal recessive. The causative genetic variant is a 4 bp deletion in the coding sequence of the FOXN1 gene encoding a developmental transcription factor. The genetic variant is officially designated as c.1030_1033delCTGT; p.L344Gfs*203.

Variants in FOXN1 also cause “T-cell immunodeficiency, congenital alopecia, and nail dystrophy” in humans and are responsible for the phenotypes seen in the nude mouse and rat mutants. In all known FOXN1 mutants, the phenotype is characterized by an almost complete absence of hair and a severe immunodeficiency.

The hereditary defect in the Birman cats has already been recognized in the 1980s (Hendy-Ibbs, 1984; Bourdeau et al. 1988; Casal et al. 1994). The clinical phenotype is characterized by an almost complete absence of hair and wrinkled skin at birth. Later on, affected kittens develop a very sparse fur with attenuated whiskers. So far, all known affected kittens have died due to infections of the respiratory organs or intestinal tract before the age of eight months (Abitbol et al. 2015).

Breeders should be counseled to test their breeding animals and to plan their matings in such a way that at least one parent is clear for this genetic defect. If breeders follow this counsel, no further affected kittens homozygous for this particular genetic defect should be born.

Hairlessness in the Sphynx cat (OMIA 001583-9685)
The hairlessness in Sphynx cats is a breed defining trait and not considered a genodermatosis by the breeders. The mode of inheritance is monogenic autosomal recessive. The causative genetic variant is a splice site variant in the KRT71 gene encoding keratin 71, which is officially designated as c.816+1G>A; r.[816+1_816+43ins; 816+1g>u] (Gandolfi et al. 2010).
Curly hair in Devon Rex and Selkirk Rex cats (OMIA 001581-9685 & 001712-9685)
The so-called rex phenotype in cats is characterized by curly hair instead of the normally straight hair. This is also not considered a genodermatosis by the breeders and represents a breed-defining trait in the Devon Rex and Selkirk Rex cats. Interestingly, their curly hair is caused by variants in the \textit{KRT71} gene, the same gene which is mutated in the hairless Sphynx cat. Currently, two different independent \textit{KRT71} variants leading to curly hair are known. Devon Rex cats are homozygous for a recessive allele with a complex genomic rearrangement (c.1108-4_1184del81insAGTTGGAG; r.1108_1221del). Cats that are compound heterozygous with one copy of the Devon Rex allele and one copy of the Sphynx hairless allele are hairless and resemble Sphynx cats (Gandolfi et al. 2010). The Selkirk Rex allele is due to the dominant c.445-1G>C; r.445_464del variant in the \textit{KRT71} gene (Gandolfi et al. 2013a). Curly hair in dogs is also caused by a \textit{KRT71} gene variant, but the known dog curly allele is due to the replacement of a single amino acid in the keratin protein rather than the complex frameshift and splicing variants seen in cats.

Curly hair in Cornish Rex and German Rex cats (OMIA 001684-9685)
Although phenotypically very similar to the above mentioned rexoid cats, the curly hair in Cornish Rex and German Rex cats is caused by a genetic variant in the \textit{LPAR6} gene encoding the lysophosphatidic acid receptor 6. The variant (c.250_253_delTTTG; pF84Efs*9) causes a frameshift in the open reading frame and leads to a recessive allele (Gandolfi et al. 2013b).

Hair length (OMIA 000439-9685)
Similar to many other species including dogs and mice, recessive loss-of-function variants in the feline \textit{FGF5} gene encoding fibroblast growth factor 5, lead to long hair in cats. For genetic testing, it has to be considered that four different \textit{FGF5} alleles are known. Any genotype involving 2 of these four loss-of-function alleles, either in homozygous or compound heterozygous state, will result in a long-haired cat (Kehler et al. 2007).

Genodermatoses & Hair Morphology Traits with Unknown Genetic Etiology
Nasal crusts in the Egyptian Mau, heritability and mode of inheritance unknown
American Wirehair cat (curly, very dense hair), autosomal dominant
Kohana (small hair follicles, no whiskers), autosomal dominant
LaPerm (curly hair), autosomal dominant
Peterbald (hairless with curly whiskers), autosomal dominant
Tennessee Rex (wavy long hair), autosomal recessive

References

UPDATE ON FELINE IMMUNE-MEDIATED SKIN DISEASES

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Introduction

Autoimmunity is a multifactorial event resulting in the breach of self-tolerance and induction of an immune response against self-antigens. In antibody-mediated diseases where autoantibodies drive the clinical phenotype of the disease, the breach in tolerance will result in the activation of self-reactive T cells that assist B cells to produce autoantibodies. Secreted autoantibodies may target specific autoantigens and cause direct tissue damage (e.g. pemphigus). Activated B cells augment inflammation via cytokine production and importantly they internalise self-antigens from damaged tissues and present them back to cognate T cells, thus amplifying the pathologic immune response. The first section of this lecture will provide a review and update of feline pemphigus foliaceus.

In some autoimmune diseases, self-reactive T cells that have lost tolerance are the main effector mechanism responsible for the clinical signs. The tissue damage seen in such disease is predominantly the result of a direct cytotoxicity of the autoreactive T cells and/or by their soluble products. The second part of this lecture will focus on thymoma-associated autoimmunity in cats and the associated clinical syndromes.

Pemphigus Foliaceus

The pemphigus group includes autoimmune blistering skin disease in which autoantibody-targeted desmosomes lose adhesion and form blisters. In several variants of pemphigus, separated according to clinical signs, the depth of acantholysis and the targeted antigen are recognised in humans and animals. Two pemphigus variants have been described in cats; pemphigus foliaceus (PF) and pemphigus vulgaris (PV).

Pathogenesis: The target antigen of autoantibodies in human PF is a desmosomal cadherin, desmoglein 1 (Dsg1). Little is known about the pathogenesis of feline PF and the target antigen(s) in cats remains unknown. There is some evidence that cats with pemphigus produce antikeratinocyte antibodies. Keratinocyte-bound IgG have been detected in a majority of feline PF and PV patents. Detection of circulating autoantibodies by indirect immunofluorescence has been demonstrated in a significant number of cats with PF.

Signalment: The age of onset of feline PF ranges from 3 months to 17 years with a median age of onset of 6 years. A sex predisposition is not reported. A breed predisposition for feline PF is not definitive.

Clinical signs: In most cats, lesions initially appear on the face, principally on the dorsal muzzle, nasal planum, periorbital skin and ears. In these areas, the pattern usually is strikingly bilateral and symmetrical. In the largest case series, the most commonly affected site was the head or face in 45 of 57 cats (78.9%) with the pinnae involved in 39 cases (68.4%) (Preziosi et al 2003). Lesions have a striking predilection for involvement of the footpads or ungual folds of claws and
can be restricted to these locations. Some cats with footpad lesions are lame with erythematous swelling at the pad margins with fissures, crusting and hyperkeratosis of pads. The subungual region is frequently involved with erythema, swelling, erosion and crusting paronychia within the nail fold characterised by green-yellow to cream-coloured, inspissated, thick exudate. The dorsum and ventrum are commonly affected, being reported in 26 (45.6%) and 22 (38.6%) of 57 cases, respectively (Preziosi 2003). Periareolar involvement occurs in approximately 10% of cats with PF. Of note is that mucosal lesions are not observed.

Skin lesions are typically transient, superficial pustules that evolve rapidly into erosions and yellow crusts with associated scale and exfoliation with alopecia. Pustules are extremely transient; however, and the phenotype is dominated by erosions and yellowish crusts. In cats with claw fold involvement, paronychia with purulent exudate is observed.

Other clinical signs include pruritus which is noted as mild to severe in some cats whereas systemic symptoms consisting of anorexia, depression, fever and weight loss are encountered usually in cats with widespread lesions.

Diagnosis

Cytology: Sample intact non-follicular pustules or remove crusts and impress a glass slide onto moist erosive surface. Cytology often reveals numerous non-degenerate neutrophils, rarer eosinophils and numerous acantholytic keratinocytes in large clusters or rafts of free-floating rounded keratinocytes. Acantholytic keratinocytes exhibit either microscopic characteristics of normal differentiated spinous or granular layer epithelial cells, or they present with signs of apoptosis with eosinophilic cytoplasm, condensed chromatin or karyorrhexis. Occasionally, neutrophils can be seen in close apposition to detached keratinocytes.

The presence of acantholytic keratinocytes and neutrophils is not specific for PF. Similar microscopical findings have been found in canine and equine cases of pustular dermatophytosis, a PF mimicker in which *Trichophyton* sp fungi invade the stratum corneum and induce subcorneal acantholytic neutrophilic pustules. Keratinocyte acantholysis has been reported also in dogs and observed in cats with bacterial skin infections.

Histopathology: Very early lesions may be vesicles with acantholytic keratinocytes and scarce neutrophils. However, these lesions rapidly evolve into broad, discrete, intragranular or subcorneal pustules with isolated and/or clustered acantholytic cells. In these lesions, neutrophils predominate, but variable numbers of eosinophils may be found. Pustules commonly invade the epithelium and/or the lumen of the follicular infundibulum. In general, the pustules are large and span the length of multiple follicular units, a finding that differentiates these lesions from those of bacterial folliculitis. Similarly, recornification, defined as newly reformed stratum corneum at the base of neutrophilic pustules, is more suggestive of PF than bacterial folliculitis. Free floating “rafts” of partially adherent acantholytic cells and adherence of acantholytic cells to the overlying stratum corneum are both characteristic histopathologic features of PF.

Direct immunofluorescence (DIF): DIF or immunoperoxidase have been used to detect antikeratinocyte autoantibodies deposited in vivo in the skin of animals with PF. Skin-fixed intercellular epidermal IgGs are detected in most cats with PF. Direct IF testing of skin biopsy
specimens from confirmed canine PF patients can be negative due to glucocorticoid therapy administered prior to specimen collection and intercellular epidermal IgG also can be found in biopsy specimens obtained from dogs with dermatoses other than PF. These findings markedly reduce the specificity of direct IF testing for the diagnosis of PF. There is no published information available for cats.

**Indirect immunofluorescence (IIF):** IIF demonstrates circulating IgG4 and IgG1 anti-keratinocyte autoantibodies in the sera of patients with PF. IIF results seem to vary according to the substrate utilized for autoantibody detection. Using neonatal mouse skin as the substrate, circulating antikeratinocyte IgG4 antibodies have been detected in the majority of dogs with PF and these antibodies are pathogenic with passive transfer into mice, causing vesiculation at epidermal levels similar to those of the natural disease. In addition, IgG4 autoantibody titres decreased during remission due to immunosuppressive therapy in some dogs with PF suggesting that monitoring titres could be useful for assessment of clinical outcome.

Detection of circulating antikeratinocyte autoantibodies by IIF has yielded only rare positive results in cats and this technique has been considered unreliable; probably due to the type of substrate used to perform the test. More recently the use of feline footpad and buccal mucosa has increased the level of detection of antikeratinocyte IgG antibodies from sera from cats with PF. (Bizikova 2015)

**Management:**
There are no established treatment guidelines for the treatment of feline PF and therefore the choice of treatment is based on published PF cases and other immune mediated diseases.

**Glucocorticoids:** Traditionally, an immunosuppressive dose of prednisolone at a dosage of 2 to 6 mg/kg orally q 24 hrs is prescribed to treat feline PF. The induction dose should be maintained until the disease is inactive, though alopecia and residual crusts may be present. Following induction, reduction of the dosage to the lowest effective and safest alternate day dosage is required for a maintenance dose. In most reported cases, a monotherapy with glucocorticoids was sufficient to control clinical signs. In cats that fail to respond to prednisolone, either dexamethasone 0.2 to 0.4mg/kg orally q 24 hrs or triamcinolone 0.6 to 2mg/kg orally q 24hrs may be a useful alternative.

**Other immunosuppressive agents:** If additional immunosuppression is required, chlorambucil at 0.2mg/kg q 24hrs for four consecutive days each week is a safe and reliable adjunctive treatment; complications to treatment are unusual but can include myelosuppression and mild gastrointestinal signs. Azathioprine has been used in feline PF but has been associated with rapid and severe bone marrow suppression and the drug is not currently recommended for cats.

More recently, it has been demonstrated that ciclosporin at a median dose of 5.5mg/kg orally q 24hrs can be both effective as a monotherapy for both remission induction and/or long term disease control as well as an adjunct therapy. Ciclosporin may provide enhanced glucocorticoid sparing for long term maintenance of cats with PF as many cats maintained with ciclosporin can be successfully withdrawn from oral glucocorticoids. (Irwin 2012)
New treatment options, whose efficacy for the management of feline autoimmune diseases awaits investigation include mycophenolate mofetil, leflunomide and methotrexate. Other options for therapy could include plasmapheresis or intravenous immunoglobulin therapy. All these drugs are used to manage other immune mediated feline diseases and may present a therapeutic option in refractory cases, especially as glucocorticoid-sparing agents.

Monitoring
There are no established guidelines for monitoring cats receiving treatment for PF; current recommendations are to perform complete blood counts and serum biochemistry with urinalysis one and three months after the onset of induction therapy and every six to 12 months for cats receiving maintenance treatment.

**Thymoma Associated Syndromes in Cats**

In cats, thymomas are an uncommon neoplasia. The most common clinical signs are dyspnoea and pleural effusion, but myasthenia gravis and thymoma-associated exfoliative dermatitis (TAED) are commonly associated entities. A case of exfoliative disease with erythema multiforme and toxic epidermal necrolysis-like disease as well as paraneoplastic pemphigus have been also described in cats with thymoma.

The thymus plays multiple roles in the development and regulation of the adaptive immune system including: (i) the development of immunocompetent T cells, (ii) differentiation of T cells into subsets, (iii) establishment of immune tolerance and (iv) regulation of production of mature T cells. It is not fully understood how a thymoma leads to immune dysregulation but several hypotheses have been proposed: (i) an escape of thymoma-driven thymocytes into the periphery without critical selection and maturation, (ii) an increased proliferation of thymocytes leading to expression of aberrant (autoreactive) T cell receptors or (iii) genetic mutations resulting in impaired expression of HLA class II molecules by epithelial cells in the thymus.

**Thymoma-associated exfoliative dermatitis syndrome**

i) Clinical presentation: erythema, exfoliation and secondary alopecia.

ii) Histopathology: epidermal hyperkeratosis, interface dermatitis and mural folliculitis; milder transepidermal apoptosis than erythema multiforme.

**Thymoma-associated exfoliative dermatitis/erythema multiforme syndrome (Godfrey 1999)**

i) Clinical presentation: erythema, exfoliation affecting the head and pinnae; erythematous papules and target lesions affecting the axillae and groin.

ii) Histopathology: keratinocyte apoptosis at all levels, lymphocyte satellitosis and interface dermatitis.

**Paraneoplastic pemphigus (PNP) (Hill 2013)**

i) Clinical presentation: maculo-papular rash progressing to erosions and ulcers affecting the ventral abdomen and external ear canals.

ii) Histopathology: subepidermal clefting with scattered apoptotic keratinocytes throughout epidermis and interface dermatitis.
Toxic epidermal necrolysis (TEN)-like syndrome (Bizikova 2015)
i) Clinical presentation: large macules progressing into ulcers affecting >70% of the body, oral and mucocutaneous ulceration
ii) Histopathology: diffuse devitalisation of the epidermis, lymphocyte exocytosis and apoptosis at all levels at the periphery; same process affects the hair follicle epithelium.

Treatment
Treatment should be aimed at removing the thymoma. In some cases; however, the autoimmune response requires an additional immunosuppressive treatment: it has been proposed that immunosuppressive doses of glucocorticoids with or without concurrent cytotoxic drugs such as chlorambucil or hIVIG (human intravenous immunoglobulin) in cases such as TEN or PNP as an initial treatment may be indicated. In cases of complete tumour removal, the requirement for immunosuppression should be temporary.

Selected References
Papillomavirus Infection

Seven different feline papillomaviruses (PV) have been identified and fully sequenced, three of them in domestic cats. The great majority of clinical cases in domestic cats are associated with FdPV2.

The first clinical signs of this infection are the so-called viral plaques. They appear as multicentric scaly papules or plaques (less than 8mm in diameter) that may be hyperpigmented, especially in the late stage of development. These lesions usually develop on the face and/or limbs. Generalization may occur. It is purported that these viral plaques are the precursory lesions of the so-called Bowenoid in situ carcinomas or BISCs. BISCs look classically like viral plaques but may be larger and more crusty. They are usually hyperpigmented. The clinical diagnosis is not always obvious and histopathological examination is mandatory to confirm the diagnosis. The viral etiology may be confirmed via PCR or immunohistochemistry even though the histological changes are usually very suggestive of a virus-induced condition. No large studies on the treatment of these lesions have been conducted but numerous anecdotal reports support the use of imiquimod for the treatment of this condition. Improvement usually occurs within three to four weeks but recurrences are often observed after treatment discontinuation. Typical warts and cutaneous horns have also been described in association with PV infections in cats but are probably very rare.

A counterpart of equine sarcoid is uncommonly observed in cats. These sarcoids are usually nodular and occur mainly on the face. Similar to equine sarcoids, these are caused by bovine papillomaviruses. It is important to understand that these sarcoids result from an infection of dermal fibroblasts and not from epidermal keratinocytes like classical PV infections.

Cowpoxvirus Infection

Feline cowpox infection is an uncommon condition of outdoor cats. In some specific areas, the condition may be more frequent. Almost all cases have been described in animals in contact with rodents and/or cattle. The primary lesion is usually a small, solitary ‘pock’ lesion occurring on the face or limbs. This early stage is usually associated with fever and anorexia. Within a few days to weeks, affected cats develop more widespread lesions, presenting as nodules, erosions, abscesses or sometimes cellulitis. During this generalized stage of the disease, some other organs may be affected, especially the lungs in immunosuppressed animals, leading to life-threatening pneumonia. The diagnosis is usually made with histological examination demonstrating the typical epidermal hyperplasia and intracytoplasmic pink inclusions. The affected individuals do not need any specific treatment and any use of immunosuppressive drugs should be avoided. This point is important because some affected cats may look like allergic patients.
**Feline Herpesvirus-1 Infection**

FHV-1 infection in cats is usually associated with conjunctivitis, rhinitis and/or stomatitis. The active stage of the infection is followed by a latent phase, where the virus persists in the trigeminal nerve. In some rare cases, reactivation of the infection may result in skin lesions, associated or not with the more classical clinical signs. Herpes dermatitis affects almost exclusively the face and is characterized by erosions, crusts and ulcerations. Interestingly, the first lesions often occur between the eye external canthus and the nose or lip area. At this stage, the lesions may be unilateral. Generalization often occurs within a few days. These lesions may be strongly pruritic and may mimic those of the so-called head and neck pruritus. For this reason, one of the most important differential diagnoses of this condition is allergic dermatitis. This is important to keep in mind because drugs such as glucocorticoids may be contraindicated in FHV-1 infected cats.

The diagnosis of this condition is often difficult and is based on the exclusion of resembling diseases, examination of skin biopsies and PCR analysis. Histopathologic changes may be easy to interpret, especially when typical intranuclear inclusions are seen. However, they may also be misleading when only necrotizing eosinophilic dermatitis is present. In this case, the main differential diagnosis is a hypersensitivity disorder. The interpretation of PCR results should also be done with caution. Negative PCR results usually indicate that a herpesvirus infection is not involved. On the other hand, positive PCR results may be due to true skin infection or due to contamination from infected mucosa through licking.

The treatment is based on the combined use of famcyclovir and L-Lysine. Some other antiviral drugs have been used but data are less convincing.

**Calicivirus Infection**

This viral infection is also associated with conjunctivitis and stomatitis, and rarely with skin changes. Caliciviruses are RNA viruses and mutations may occur quickly. That is probably the reason why some less classical syndromes associated with this virus have been described in the last few years. One of these syndromes involves only the skin and affects the face and feet. Affected animals usually present with fever, swollen feet and erosive changes on the face, especially the nose. This episode usually lasts a few days and is followed by a spontaneous resolution of the clinical signs.

**FeLV Infection**

Some classical FeLV infections in cats may lead to skin changes. The lesions may be seborrheic or ulcerative. The diagnosis is made with histopathology showing typical giant keratinocytes and positive immunohistochemistry. Some cutaneous horns have also been seen in FeLV-infected patients even though causality has not been proved.

**FIV Infection**

This lentivirus does not induce specific skin changes but leads to severe immunodeficiency. This situation may favor the development of opportunistic infections with microbes such as staphylococci, mycobacteria, dermatophytes, yeast or *Demodex* mites.
Selected References

DIAGNOSIS AND TREATMENT OF FELINE RESPIRATORY DISEASE WITH SKIN MANIFESTATIONS

Angie Hibbert

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1. Introduction
   The spectrum of signs associated with respiratory disease in cats is wide, ranging from those presenting in an acute dyspnoeic crisis to those with only subtle or intermittent mild signs such as cough or increased expiratory effort due to inflammatory lower airway disease. A logical and systematic approach is required to identify and investigate feline respiratory disease. It is critical to remember that a dyspnoeic cat has limited reserve and excessive handling, restraint or stress may precipitate respiratory arrest.

2. Diagnostic approach
   A thorough history and physical examination is essential for any medical investigation, and features pertinent to respiratory disease will be highlighted in this lecture. Presenting signs and physical abnormalities should be used to try to localise which area of the respiratory system is affected. It is helpful to consider the respiratory system divided into five regions:
   - Upper airways (upper respiratory tract - URT) - nose, pharynx, larynx and trachea
   - Lower airways (lower respiratory tract - LRT) - bronchial tree
   - Pulmonary parenchyma
   - Pleural space and mediastinum
   - Thoracic wall – ribs, intercostal muscles and diaphragm

A. History
   In addition to a standard dermatological history, the following questions should be addressed when evaluating for respiratory disease
   i.) Any prior history of respiratory disease? This is especially important when considering whether the cat could be a carrier of feline herpes or calicivirus
   ii.) Any recent coughing, gagging or retching? Coughing is frequently misinterpreted as vomiting by owners if the cat gags or has a terminal retch associated with it
   iii.) Any recent audible respiratory noise - stertor, stridor, wheezing?
   iv.) Any dysphonia or loss of miaow? If yes, this may indicate laryngeal disease
   v.) Any nasal or ocular discharge? Any other cats in the household with similar signs? If yes, infectious causes are more likely
   vi.) Any recent vomiting or regurgitation? If yes, consider the possibility of aspiration pneumonia
   vii.) Rate of onset of signs?
   viii.) Any signs of systemic disease, e.g. recent weight loss, polyuria/polydipsia, polyphagia, and inappetence?
   ix.) Any known history of trauma or toxin exposure, e.g. rodenticide?
   x.) Any observed changed in activity levels?
B. Physical examination
Examination is best performed in a quiet environment with minimal patient restraint. In the acutely dyspnoeic patient a limited assessment may be possible, whilst oxygen is administered; in this situation the initial assessment should prioritise cardiorespiratory system examination, with completion of a full physical exam once the patient has been stabilised.

i.) First observe the cat’s breathing pattern and rate hands-off. A normal respiratory rate is 15-30bpm. The thoracic and abdominal wall should move synchronously with minimal effort, moving outwards on inspiration and inwards on expiration. The length of inspiration and expiration should be similar.
   a. Dyspnoea: difficulty in breathing; often manifests in cats as open-mouth breathing.
   b. Tachypnoea: increased rate of breathing (>30 breaths per minute).
   c. Hyperpnoea: increased depth of breathing, with or without an increase in the rate of breathing.
   d. Orthopnoea: adoption of a standing or sternal position to aid respiration.
   e. Paradoxical respiration: loss of co-ordination between thoracic and abdominal wall movements (may be a sign of respiratory muscle weakness or fatigue).

Depending on where the compromised area of respiratory tract is, the cat will adapt its breathing pattern by altering either the rate or depth of breathing, in an attempt to maintain oxygen intake. Identifying these changes and patterns can help localise the affected area. Table 1 provides details about the assessment of respiratory patterns.

ii.) Assess for the presence of any respiratory noise: auscultate over the URT (larynx and trachea) and then auscultate the entire thorax using a grid system to help identify differences between regions of the pulmonary fields.

iii.) Assess for changes on percussion of the thorax: percuss the entire thorax using the grid system to identify areas with altered resonance. Fluid and masses will decrease the resonance, making a dull sound; free air will increase thoracic resonance and sound more tympanic.

iv.) Assess for the presence of any nasal or ocular discharge.

v.) Check airflow through the nares – use a microscope slide to look for exhaled air condensation.

vi.) Assess mucous membrane colour and capillary refill time: look for pallor, cyanosis, congestion, petechiation and ecchymoses.

vii.) Check peripheral and femoral pulse quality and rate.

viii.) Check the position of the apex beat, e.g. has it moved due to a thoracic mass?

ix.) Auscultate the heart for one minute (minimum) listening for arrhythmias, a gallop rhythm, or a heart murmur and palpate the femoral pulse simultaneously (to help identify any ‘dropped’ beats).

x.) Check for jugular pulses, which are a sign of raised systemic venous pressure.

xi.) Palpate for any obstructive masses over the larynx and trachea.

xii.) Assess for increased tracheal sensitivity - cough induced by gently squeezing the trachea ‘tracheal pinch’.

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xiii.) Check the cranial rib spring; increased resistance is usually consistent with a cranial mediastinal mass.

xiv.) Assess rectal temperature; hyperthermia may develop with increased respiratory work.

xv.) Complete remainder of general physical examination if the patient is cardiovascularly stable.

Several diseases may have respiratory and dermatological manifestations particularly infectious diseases including feline herpes virus infection, feline calicivirus systemic virulent disease, cowpox and mycobacterial disease, along with neoplasia. The remainder of these notes will focus on identification and treatment of inflammatory lower airway disease and systemic anaphylaxis, which may be potential emergency pathologies.

3. Feline bronchial disease – inflammatory lower airway disease

Asthma and chronic bronchitis are both types of inflammatory bronchial disease and it is likely that they are two forms of a heterogeneous group of airway diseases. Asthma is thought to be due to a Type 1 hypersensitivity response to inhaled allergens, resulting in spontaneous and reversible bronchoconstriction, eosinophilic airway inflammation and remodelling. Chronic bronchitis is characterized by neutrophilic airway inflammation and remodelling; it is not typically associated with spontaneous bronchoconstriction and does not have an allergic basis. Chronic bronchitis is a diagnosis of exclusion; infectious disease and neoplasia have to be ruled out. Distinguishing asthma from chronic bronchitis without examination of airway cytology is challenging however having an awareness of the possible existence of lower airway disease may be of specific importance to the dermatologist; an undiagnosed asthmatic cat undergoing intradermal skin testing or immunotherapy may potentially be at risk of anaphylaxis with acute bronchoconstriction and respiratory crisis. From a therapeutic perspective, asthmatic cats may benefit more from bronchodilator therapy than cats with chronic bronchitis and in the future, distinguishing asthma from chronic bronchitis may be more important if treatment can be directed towards the underlying allergy.

A. Clinical features

Asthma is more commonly diagnosed in young adult cats, with Siamese and Oriental breeds predisposed. Chronic bronchitis may develop at any age, and there are no known breed predispositions.

Chronic bronchitis is associated with a frequent harsh cough. Often coughing is misinterpreted as vomiting, since many cats will terminate a paroxysm of coughing with a retch; owners frequently present the cat for hairballs. Asthma may also cause coughing, which may be paroxysmal, daily or intermittent, as the disease can wax and wane. There may also be associated lethargy, exercise intolerance and episodes of dyspnoea due to acute bronchoconstriction, with open-mouth breathing, tachypnoea, wheezing, pallor, cyanosis and collapse. Physical examination may reveal harsh respiratory sounds, wheezes/crackles and increased expiratory effort with an abdominal ‘push’. The thorax may be hyper-resonant in asthmatic cats, due to chronic pulmonary over-inflation. Auscultation of the asthmatic cat between episodes of bronchoconstriction may be completely normal.
B. Diagnosis

i.) Thoracic imaging (Radiography or CT) is usually performed before obtaining airway washes.
   a. Asthmatic cats may have variable pulmonary patterns or even normal thoracic radiographs.
   b. The most common feature of inflammatory lower airway disease is a bronchial pattern, with bronchial wall thickening and mineralisation (‘tramlines and doughnuts’ on radiographs).
   c. Interstitial and focal alveolar radiographic patterns may also be seen, due to airway obstruction by mucus plugs, causing local atelectasis; the right middle lung lobe is most often affected.
   d. Asthmatic cats may have pulmonary hyperinflation causing flattening of the diaphragm; there may be evidence of previously healed proximal rib fractures in the caudal thorax. Rarely spontaneous pneumothorax may occur as a result of small airway obstruction causing increased alveolar pressures and emphysema.

ii.) Airway sampling
   a. Cytology of airway washes is the basis for diagnosing asthma or chronic bronchitis. Bronchoalveolar lavage (BAL) samples can be obtained blindly or at bronchoscopy, once the cat is stable enough to undergo general anaesthesia. Chronic bronchitis is characterized by neutrophilic inflammation, and asthma by eosinophilic inflammation (>25% of the total cell count).
   b. Bacteriology: Microbial culture of BAL samples should be performed (for aerobic, anaerobic and Mycoplasma spp.) +/- PCR for Mycoplasma felis and Bordetella bronchiseptica to identify concurrent airway infection.

iii.) Additional diagnostic tests
   a. Haematology: An inflammatory leucogram may be identified; peripheral eosinophilia is an inconsistent finding in asthmatic cats.
   b. Serum biochemistry: Rarely helpful; hyperglobulinaemia may be identified.
   c. Faecal parasitology: Faecal flotation and Baermann technique to search for Aelurostrongylus abstrusus and Eucoleus aerophilus.
   d. Serology: For FeLV and FIV.
   e. Dirofilaria immitis serology: in endemic areas.

C. Management

i.) Emergency treatment of the dyspnoeic asthmatic cat
   a. Provide supplemental oxygen (oxygen cage, flow by if tolerated, oxygen hood).
   b. Administer a bronchodilator (terbutaline 0.015 mg/kg IM or IV; dose can be repeated after 30-60 minutes if partial response only, then q4-6h as required) and establish intravenous access if not already present. Inhalant bronchodilators (albuterol (salbutamol) at 100 mcg/actuation by metered dose inhaler) can be used however administration may be more stressful for an acutely dyspnoeic cat.
   c. Administer a short-acting corticosteroid (e.g. dexamethasone sodium phosphate 0.1-0.2 mg/kg IV or SQ once, or hydrocortisone 2 mg/kg IV q6h).

ii.) Longer-term management
Chronic bronchitis and asthma are both treated with anti-inflammatory steroids, ideally delivered by inhalation to reduce systemic absorption.

a. Fluticasone propionate - 125-250 µg (micrograms)/cat, one dose q12h, with gradual introduction of the spacer device (Aerokat™), to help compliance. Fluticasone typically reaches therapeutic levels after 2 weeks, therefore oral prednisolone is started simultaneously and tapered off once therapeutic levels of fluticasone are achieved: e.g. 0.5 mg/kg orally q12h for two weeks, then 0.5 mg/kg orally q24h for one week then 0.5 mg/kg orally on alternate days for seven doses and then stop. The dose of inhaled steroid is gradually tapered after a period of stability (minimum 2-4 weeks); it is important to remember, however, that inadequate treatment of airway inflammation can be detrimental, due to ongoing airway remodelling.

b. Concurrent airway infection should be excluded - on the basis of either airway culture or therapeutic trials (e.g. doxycycline at 10 mg/kg orally q24h for 3-6 weeks to exclude *Mycoplasma* spp. infection; topical imidacloprid/moxidectin or oral fenbendazole at 50 mg/kg q24h for 10 days to exclude lungworms).

c. Asthmatic cats may benefit from continued use of a bronchodilator until the disease stabilizes; terbutaline and theophylline are both available in an oral form. Terbutaline and inhaled albuterol are both reserved for acute disease (racemic formulation of albuterol may have some pro-inflammatory effects).

d. Advice to improve airway hygiene is essential; avoidance of exposure to smoke, aerosols, air fresheners and dust from litter trays, and restrict the cat from the bedroom.

e. Steam nebulization may help to improve airway moisture levels and mucociliary clearance. Some cats may benefit from mucolytic treatment (e.g. bromhexine or acetylcysteine).

4. **Feline systemic anaphylaxis**

   Is an acute life threatening allergic reaction with numerous possible triggers including ‘allergens’ used for intradermal testing and drugs. In the cat, acute respiratory signs are more often observed with laryngeal oedema and bronchoconstriction causing dyspnoea. Details of emergency management are provided in table 2.
Table 2. Feline systemic anaphylaxis – management

**Signs:** hypotension, laryngeal and pharyngeal oedema, bronchospasm, urticaria, erythema, angioedema, arrhythmias, hyper-peristalsis, vomiting and diarrhea

<table>
<thead>
<tr>
<th>Management</th>
<th>Treatment(s)</th>
<th>Purpose/effect</th>
<th>Additional information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Airway management</strong></td>
<td>Supplemental oxygen; flow by or intubation</td>
<td>Maintain oxygen delivery</td>
<td>Aim for SpO₂ &gt;95%; ventilation may be required</td>
</tr>
<tr>
<td><strong>Vascular access and intravenous fluid therapy</strong></td>
<td>Crystalloids initially (5-10ml/kg boluses IV over 10mins to 60ml/kg total dose); colloids if refractory hypotension</td>
<td>Maintain perfusion (distributive shock and relative hypovolaemia)</td>
<td>Care with fluid loading in cats; monitor heart rate and for the development of gallop rhythms</td>
</tr>
<tr>
<td><strong>Epinephrine</strong></td>
<td>0.01mg/kg IM (1:1000 solution=1mg/ml; maximum dose 0.3mg &lt;40kgs); 0.05µg/kg/min slow IV (CRI) if established shock &amp; titrate to effect</td>
<td>Inotropic, chronotropic effect, bronchodilation, peripheral vasoconstriction, reduce mucosal oedema</td>
<td>Treatment of choice for anaphylaxis; monitor heart rate, rhythm and blood pressure; side – effects include ventricular arrhythmia, hypertension, pulmonary oedema</td>
</tr>
<tr>
<td><strong>Ancillary treatments</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Glucocorticoids</strong></td>
<td>0.1-0.2mg/kg dexamethasone sodium phosphate IV</td>
<td>Decreased production inflammatory mediators</td>
<td>Not a first line treatment; effects will be delayed (hours)</td>
</tr>
<tr>
<td><strong>Anti-histamines</strong></td>
<td>H1 diphenhydramine 1mg/kg IV, IM</td>
<td>H1 blockade -reduce pruritus &amp; angioedema</td>
<td>Evidence base lacking-symptomatic treatment</td>
</tr>
<tr>
<td></td>
<td>H2 ranitidine 2.5mg/kg IV (slow), PO, SC</td>
<td>H2 blockade -reduce histamine effect on the gastro-intestinal tract</td>
<td></td>
</tr>
<tr>
<td><strong>Bronchodilators</strong></td>
<td>Terbutaline 0.015mg/kg IV, IM; albuterol (salbutamol) 100 mcg/actuation by metered dose inhaler</td>
<td>Reverse acute bronchoconstriction</td>
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</tbody>
</table>
Table 1. Assessment of respiratory patterns.
Adapted from the BSAVA Manual of Feline Practice, with permission

<table>
<thead>
<tr>
<th>Area of the respiratory tract</th>
<th>Affected phase of respiration</th>
<th>Breathing pattern</th>
<th>Features</th>
<th>Potential localizing sounds</th>
<th>Differential diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary parenchyma</td>
<td>Inspiration ± expiration: increased resistance to lung inflation on inspiration ± reduced compliance on exhalation</td>
<td>Restrictive pattern</td>
<td>Shallow rapid breathing. Length of inspiratory phase = expiratory phase</td>
<td>Crackles (see above) or wheezes (see above) over pulmonary fields. History of soft, moist cough</td>
<td>Pulmonary oedema: cardiogenic; non-cardiogenic; hypoalbuminaemia, smoke inhalation; near drowning; SIRS; electrocution; cervical or head trauma; seizures. Bronchopneumonia. Haemorrhage: coagulopathy; contusions; neoplasia. Thromboembolism. Acute lung injury / acute respiratory distress syndrome. Idiopathic pulmonary fibrosis</td>
</tr>
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<td>Area of the respiratory tract</td>
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<tr>
<td>Pleural space and mediastinum</td>
<td>Inspiration: increased resistance to lung inflation</td>
<td>Restrictive pattern and/or increased abdominal effort. Paradoxical pattern possible</td>
<td>Shallow rapid breathing. Length of inspiratory phase = expiratory phase. Exaggerated abdominal effort. Paradoxical pattern possible; inward movement of the abdominal wall during inspiration</td>
<td>Loss of lung sounds dorsally if pneumothorax. Loss of lung sounds and muffled heart sounds ventrally if pleural effusion or mediastinal mass. Possible audible intestinal sounds over thorax if diaphragmatic rupture</td>
<td>Effusion: transudate – hypoalbuminaemia; modified transudate - CHF, neoplasia, lung lobe torsion; exudate - FIP, pyothorax (septic); chyle - CHF, neoplasia, trauma, lung lobe torsion, idiopathic; haemorrhage - coagulopathy, trauma, neoplasia. Mass: mediastinal lymphoma, thymoma, abscess. Diaphragmatic rupture. Pneumothorax: trauma, asthma, neoplasia</td>
</tr>
<tr>
<td>Thoracic wall (ribs, intercostal muscles, diaphragm)</td>
<td>Inspiration ± expiration-impaired musculoskeletal function (combined with effect of pleural space occupying lesion if diaphragmatic rupture)</td>
<td>Paradoxical or restrictive</td>
<td>Paradoxical: loss of coordination between thoracic and abdominal wall movement. Shallow rapid respiration with progressive reduction in thoracic wall excursion. Flail chest: section of thoracic wall sucked inwards during inspiration, due to 2+ consecutive rib fractures</td>
<td>Loss of lung sounds ventrally ± audible intestinal sounds possible with diaphragmatic rupture and intestinal herniation</td>
<td>Flail chest: thoracic trauma. Diaphragmatic rupture: trauma, pericardio-peritoneal hernia. Musculoskeletal weakness: lower motor unit dysfunction (junctionopathy, neuropathy or myopathy); C1-C6 injury; occasionally a consequence of prolonged dyspnoea. Pectus excavatum</td>
</tr>
</tbody>
</table>

CHF = congestive heart failure; FIP = feline infectious peritonitis; SIRS = systemic inflammatory response syndrome.
References and further reading
CLINICAL APPROACH TO FELINE SYSTEMIC DISEASES WITH DERMATOLOGICAL MANIFESTATIONS

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Introduction
The skin functions in providing innate protection and maintaining homeostasis along with the systemic factors that can influence its integrity makes it a critical sentinel for systemic disease. Some cutaneous changes are so intimately associated with a particular underlying organ dysfunction or disorder that they are immediate visual clues to evaluate for specific diseases. The color change seen in an animal with icteric mucous membranes is a clear indicator to evaluate for causes of jaundice in that patient. Changes in the appearance of the skin may be markers of pathology occurring in another organ system or they may represent a disease process that is multi-systemic, such as seen with some infectious diseases or in systemic lupus erythematosus. Both the appearance and integrity of the skin are influenced by a number of systemic factors. These factors include the nutritional status, hormonal levels and interactions, perfusion and vascular integrity and the overall health and systemic organ function of the individual animal. Consequently changes in the skin can be a critical sentinel for systemic disease. The skin is also readily accessible for diagnostic sampling and can in some cases provide the necessary information for making the diagnosis of systemic disease. Recognizing those skin changes that are clinical markers for underlying systemic disease can expedite the diagnosis and timely management of those diseases.

Cutaneous Changes Associated with Hormonal Disturbances
Endocrine diseases provide excellent examples of the connection between disease and the skin but the most common endocrinopathies in the cat, hyperthyroidism and diabetes mellitus, often have nonspecific changes in coat quality such as, varying degrees of alopecia and disturbances in cornification due to altered metabolism or secondary to poor grooming in an unwell cat. Acromegaly from excessive growth hormone and disturbances in sex hormones, other than progesterone (see acquired skin fragility and xanthomas), rarely cause any skin changes in the cat.

Thyroid Hormone
Thyroid hormones are very important to the skin and promote the initiation of the anagen phase of the hair follicle cycle. Hypothyroidism results in disturbances in cornification, an increase in the number of hair follicles in telogen and accumulation of glycosaminoglycans in the dermis. Clinically this results in alopecia, a dull, dry hair coat, variable hyperpigmentation, scaling and myxedematous changes. In hypothyroidism, the normal barrier function of the epidermis is likely impaired and in animal models impaired neutrophil and lymphocyte function has been reported. Consequently recurrent pyoderma and otitis externa can occur. Spontaneous hypothyroidism in cats is extremely rare. One reported case had similar clinical signs to dogs including a dull, dry, haircoat that was lighter in color than normal and the cat had...
a puffy face but experimentally thyroidectomized cats did not; they reportedly groomed less, developed matting and seborrhea but only focal alopecia on pinnae and pressure points. Hyperthyroid cats can develop matting, seborrhea, increased shedding and over-grooming. With chronicity, alopecia may develop with hypotonic, thin skin.

Glucocorticoids
Excessive glucocorticoids cause cornification abnormalities, inhibit fibroblast proliferation and collagen production and cause pilosebaceous atrophy. Clinically, excessive cortisol (endogenous or exogenous) also results in disturbances in cornification, dermal thinning and delayed wound healing. Naturally occurring hyperadrenocorticism is rare in the cat and skin lesions have been seen in about half of the reported cases and include alopecia, thin skin, increased susceptibility to bruising, scaling, comedones and fragile skin.

Acquired Skin Fragility
Acquired skin fragility in cats is associated with hyperadrenocorticism (often adrenal tumors), iatrogenic hyperglucocorticoidism, or excessive levels of progestational compounds from either adrenal tumors or the iatrogenic effect of administered progestational compounds. Affected cats have extremely thin, fragile skin that easily bruises and can be torn with simple manipulations, often during restraint or handling. There are also rare reports of feline skin fragility being associated with hepatic lipidosis and hepatic neoplasia.

Cutaneous Paraneoplastic Syndromes and Metastatic Skin Disease
A paraneoplastic syndrome is defined as either a disease or clinical signs or symptoms that develop distant from the site of a tumor; it is caused by the presence of the tumor or its metastasis, but does not result from the local presence of neoplastic cells. Paraneoplastic syndromes are often mediated by hormones, cytokines or growth factors released by tumors or as an immune response targeted against the tumor. The term paraneoplastic is thought by some to be an inappropriate term to use if the clinical signs are associated with neoplastic tissue producing more of the same substance it normally produces. Consequently, diseases such as hyperadrenocorticism caused by either an adrenal tumor or pituitary tumor is not considered paraneoplastic, although some review papers may cite it as an example. Paraneoplastic skin diseases represent a group of skin disorders that if recognized should alert the clinician to underlying internal neoplastic disease. These syndromes are seen most commonly in middle-aged to elderly individuals.

Feline Paraneoplastic Alopecia
This is a rare, yet highly characteristic skin disease that occurs in association with pancreatic adenocarcinoma. Affected cats develop precipitous, ventrally pronounced alopecia in which the skin appears very shiny and smooth but is not fragile. Some cats may also have dry, exfoliative, and shiny footpads often with concentric circular rings of scale. On necropsy exocrine pancreatic adenocarcinoma with hepatic metastases is the most common tumor found but bile duct carcinoma has been reported in two cases. The disease affects older cats and the chief clinical complaint is often the acute and dramatic alopecia that affects the ventral trunk, medial aspects of the limbs and the ventral cervical region, but can generalize. Remaining hairs will epilate easily. Secondary Malassezia infections are common and may contribute to why some affected cats groom excessively potentially exacerbating the alopecia. Histopathology of a skin
biopsy reveals epidermal hyperplasia with marked follicular and adnexal atrophy. Any cat with a tentative diagnosis of paraneoplastic alopecia should undergo an abdominal ultrasound to evaluate for the presence of a pancreatic or hepatic mass. Temporary resolution of the cutaneous disease was reported in one cat after the primary pancreatic tumor had been removed; the lesions recurred with development of metastatic disease.

Feline Thymoma-Associated Exfoliative Dermatitis
A rare, exfoliative dermatitis has been described in middle aged to older cats with thymomas. The exact pathogenesis is not known but is thought to be an immunologic etiology potentially T cell mediated. Histologically, it is similar to an erythema multiforme or graft versus host type of reaction. Skin lesions tend to begin on the head and pinnae but can quickly generalize to involve the entire cat. Generalized erythema and marked scaling are present. Secondary infections with bacteria and Malassezia may develop. Respiratory signs secondary to the cranial mediastinal mass may be present at the time of presentation but in most cases skin changes precede any other systemic signs. Histopathology of representative skin lesions reveals a cell poor, hydropic interface dermatitis with apoptosis (single cell necrosis) of basal cell keratinocytes. If detected and diagnosed, removal of the thymic tumor will lead to resolution of the dermatologic clinical signs. This reaction can also be idiopathic as has also been reported in humans.

Metastatic Lesions from Pulmonary Adenocarcinoma
Primary pulmonary adenocarcinoma can in cats metastasize to the distal phalanges of digits. Cats will sometimes present with digital swelling or lameness. Typically, multiple digits on different paws are affected. Cats may have no respiratory signs so it is important to obtain thoracic films in all cats with digital lesions.

Cutaneous Manifestations of Nutritional or Metabolic Perturbations
The skin can develop lesions secondary to nutritional deficiencies, however, this is very uncommon in a patient that has a good appetite and is eating a well-balanced commercial food. Some cutaneous manifestations of nutritional deficiencies are recognized in particular breeds suggesting perhaps an alteration in absorption or metabolism while others have been linked to inadequate or unbalanced diets. Superficial necrolytic dermatitis can be a paraneoplastic skin marker if associated with glucagonoma but it is more commonly associated with some yet to be determined alterations in metabolism that causes depletion of amino acids. Underlying disturbances in lipid metabolism can result in the development of cutaneous xanthomas.

Cutaneous Xanthomas
Cutaneous xanthomas are rare and occur when there is underlying hereditary defects in lipid metabolism or acquired dyslipoproteinemia secondary to diabetes mellitus, or use of megestrol acetate. These skin lesions result from the accumulation of lipid laden macrophages within the dermis. Feline cutaneous xanthomas may develop in cats with hereditary hyperchylomicronemia, megestrol acetate induced diabetes mellitus or naturally occurring diabetes mellitus. Apparent idiopathic cases of xanthomas with no identifying underlying metabolic or hormonal disturbance have been reported. Often, affected animals are consuming a diet rich in fats or triglycerides at the time they develop lesions.
Clinically, cutaneous xanthomas present as multiple pale yellow to white plaques, papules or nodules with erythematous borders. They are often located on the head, particularly the preauricular area or pinnae. Lesions can develop in paw pads and over boney prominences on limbs. Lesions may bruise readily and larger masses may in rare cases ulcerate and exude inspissated necrotic material. Cats with inherited hyperchylomicronemia may also demonstrate peripheral neurologic signs due to nerve compression from subcutaneous xanthoma formation. Histologic evaluation of skin biopsies reveals large foamy macrophages and giant cells. Serum biochemistry evaluations for diabetes mellitus, hypercholesterolemia and hypertriglyceridemia should be obtained. Feeding of a low fat diet and identification and correction of the underlying disturbance in lipid metabolism is recommended for patients that have had cutaneous xanthomas identified.

Vitamin E deficiency: Pansteatitis
Pansteatitis is associated with diets that are low in Vitamin E and high in polyunsaturated fats. A diet comprised entirely of raw oily fish is a classic example. Cats with pansteatitis develop firm, painful swellings associated with the subcutaneous inguinal and abdominal fat pads. The swellings result from the inflammation associated with the peroxidative damage of adipose tissue. Cats may be painful and reluctant to move, anorexic or febrile. It is important to differentiate this disease from panniculitis caused by infectious agents such as the opportunistic mycobacteria that can often cause nodular lesions on the ventral abdomen. Diagnosis is made based on supportive history and histologic evidence of steatitis on biopsy. Biopsy reveals lobular panniculitis with macrophages and giant cells and there is ceroid within lipocytes. Correcting the dietary deficiency and vitamin E supplementation will improve clinical signs.

Cutaneous Manifestations of Systemic Infectious Diseases
Sometimes the skin provides valuable clues to underlying infectious disease. Skin lesions can develop in association with systemic infectious disease. In cats, systemic mycosis and a number of viral diseases can have cutaneous lesions develop as a consequence of the underlying systemic infectious disease. Sometimes, in these diseases the organism can be found within the lesional skin. Infectious diseases can also cause skin lesions as a consequence of the systemic vasculitis or thrombocytopenia that may occur in association with the infectious disease, example feline infectious peritonitis (FIP)

Systemic Mycosis
Many systemic or deep mycoses (blastomycosis, coccidioidomycosis, cryptococcosis, histoplasmosis, aspergillosis and, in some cats, sporotrichosis) can present with cutaneous lesions along with clinical signs referable to infection in other organ systems. These skin lesions include papules, nodules, draining tracts and ulceration that result from hematogenous dissemination of the fungal organism to the skin or depending on the fungal species direct inoculation of fungal organisms into a cutaneous wound. Skin lesions are seen most commonly in feline cryptococcal infections and sporotrichosis. Typically, there are other systemic clinical signs. Diagnosis of any of the fungal infections is based on demonstration of the organism within biopsied tissue and/or fungal culture. Suspicious cutaneous lesions can provide easy and rapid diagnostic information in the evaluation of animals with systemic mycoses. Appropriate antifungal therapy is chosen based on type of organism and overall health of the animal.
Systemic Viral Diseases
A number of viral diseases can in addition to systemic clinical signs also cause cutaneous lesions. These include in cats the retroviruses FeLV/FIV, feline herpesvirus, feline calicivirus and feline coronavirus causing feline infectious peritonitis (FIP). Feline papillomavirus causes cutaneous lesions and not systemic disease but they can develop more readily, become more severe or difficult to manage in patients with underlying immunosuppression.

Feline Retroviruses
Opportunistic skin infections, oral ulcerations and gingivitis have been associated with FeLV and FIV. Cutaneous horns can develop on the paw pads of cats with FeLV. In severe cases, lameness and discomfort can be marked. Diagnosis is confirmed with a positive FeLV status and skin biopsy. Immunohistochemistry can demonstrate the presence of the virus within a skin biopsy. Cutaneous lymphoma and giant cell dermatosis have also been reported in FeLV positive cats.

Feline Herpesvirus
Feline herpesvirus ulcerative dermatitis typically involves the dorsal muzzle but lesions may extend to involve the nasal planum. Cats do not have to have concurrent ocular or upper respiratory tract signs. Histologically, the lesion is a necrotizing, ulcerative dermatitis most often with a concurrent marked eosinophilic inflammation, but the inflammatory pattern may be strongly neutrophilic in some cases. The presence of eosinophilic inflammation and the clinical appearance of the lesions make it difficult to differentiate from mosquito bite hypersensitivity or other feline eosinophilic ulcerative lesions. Unless intranuclear viral inclusions can be identified it is not possible to definitively diagnose the virus as the etiologic agent for the ulcerative dermatitis. Polymerase chain reaction (PCR) has been shown to be a sensitive test to detect the presence of the virus within skin biopsies. Treatment can include subcutaneous administration of alpha interferon (1,000,000 units/m², 3 times a week), oral famciclovir (Famvir, Novartis Pharmaceuticals) (60-90 mg/kg), and/or lysine.

Vascular Disease
Vasculitis
Vasculitis can occur as a primary disease but is more commonly secondary to some other underlying disease process such as, an infectious disease, neoplasia, immune-mediated connective tissue diseases or adverse drug reactions. There are both immunopathogenic and non-immunopathogenic mechanisms that can induce vasculitis. Non-immunopathogenic mechanisms that are involved in vasculitis do so without primary attack on components of the vascular wall. These mechanisms include invasion of the vascular wall with neoplastic cells or microbial agents and influences of burn, trauma, endotoxin or hemodynamic factors on the integrity of the vascular wall. Immunopathogenic mechanisms for vasculitis include in situ formation or deposition of immune complexes, antibodies directed against vascular wall components, anti-neutrophilic antibody-mediated vessel damage, cytotoxic T cells directed against vascular components and cytokine induced mechanisms.

Vasculitis may involve only one organ system such as the skin or may involve multiple organ systems and consequently clinical signs can be variable. Cutaneous vasculitis typically results from small vessel vasculitides with lesions of swelling, erythema, hemorrhagic macules, plaques
or bullae. Ischemic necrosis and ulceration are often present in lesional areas often located on extremities or over pressure points. Foot pads if affected often have depressed areas of central pallor.

Perhaps the most important information to ascertain when evaluating a patient with vasculitis is the possibility of an underlying infectious etiology. If infectious vasculitis is not occurring, the clinician needs to evaluate for exogenous or endogenous antigens that may be triggering the disease. In one study greater than 50% of the cases were deemed to be idiopathic\(^{13}\). If an underlying antigenic drive cannot be identified, the vasculitis should be described based on pathologic evaluation of vessel type, size, location and inflammatory infiltrate. Histopathologically, vasculitis is often characterized by neutrophilic nuclear debris so called leukocytoclasia, inflammatory cells within the vessel wall, fibrinoid necrosis of vessels and extravasation of RBCs into the surrounding dermis.

Feline vasculitis has been reported as an adverse consequence of drug administration and there are reported cases implicating carbimazole, fenbendazole and oral cimetidine as drug triggers\(^{14,15}\). Viral infection with feline coronavirus progressing to FIP has been reported to cause cutaneous vasculitic lesions\(^{14}\).

Therapy is dictated by identifying underlying triggers. Infectious etiologies need to be treated appropriately, possible inciting drugs should be discontinued, underlying diseases need to be identified and immunosuppressive or immunomodulatory therapy may be warranted.

**Autoimmune Skin Diseases Associated with Systemic Disease**

Autoimmune skin diseases are uncommon skin disorders and are reported to account for less than 2% of all skin diseases seen in small animal practice\(^{16}\). They are often clinically impressive and can even be life threatening. Definitive diagnosis requires timely biopsy of appropriate representative skin lesions and cannot be based solely on clinical impression or appearance.

**Systemic Lupus Erythematosus (SLE)**

SLE is a multi-systemic autoimmune disease. Skin disease occurs variably with percentages as high as 40 to 50% of cases of SLE having skin lesions\(^{16}\). Fever, polyarthritis, protein losing nephropathy from glomerulonephritis, anemia, and thrombocytopenia are the more common clinical signs seen with SLE. Organ specific and non-organ specific autoantibodies target a variety of tissue antigens in SLE. Resultant tissue damage occurs when there is immune complex deposition (as occurs in glomerulonephritis) or can occur because of direct cytotoxic effects or cell-mediated immunity.

Cutaneous lesions are variable and can include erythema, scaling, crusting, depigmentation, alopecia and ulcerations. Lesions may be present on mucocutaneous junctions and within the oral cavity. Ulcers and erosions are rarely diagnostic lesions to biopsy as an intact epidermis is needed to make a definitive diagnosis. The histopathologic findings are variable but classic lesions include apoptosis of basal cells and basal cell vacuolation which lead to dermo-epidermal separation and consequent ulceration.
There are published criteria for the diagnosis of SLE in dogs and definitive diagnosis requires the presence of four or more criteria\textsuperscript{16}. These criteria include identification of immune-mediated disease targeting various organs systems/tissues +/- a positive ANA. It is difficult to know if these criteria could be applied to cats as there is much more limited data about this disease in this species and some published cases may be probable rather than definitive SLE. Systemic lupus erythematosus is a progressive disease and evidence of immunologic involvement in multiple organ systems may not always be evident on the initial presentation. A thorough systemic evaluation including a complete blood cell count, serum biochemistry, urinalysis, +/- protein to creatinine ratio, antinuclear antibody (ANA), arthrocentesis and evaluation of joint fluid cytologically may be indicated in patients suspected of having SLE. Most patients with SLE have an elevated ANA although this may not always be present. Prognosis depends in large part on the organ systems involved. Immunosuppressive therapy with corticosteroids with or without other immunosuppressive drugs (azathioprine [Immuran], chlorambucil [Leukeran], cyclosporine [Atopica]) is utilized.

Erythema Multiforme (EM)
The terminology has been, over the years, confusing in both human and veterinary medicine in regards to EM and Steven-Johnson’s syndrome /toxic epidermal necrolysis (SJS/TEN). It has been documented in human beings that the cell-mediated immune response in EM has a Th 1 pattern\textsuperscript{17}. The exact mechanism has not been proven in cats but a T cell-mediated response directed at keratinocytes is most often proposed resulting in apoptosis (single cell necrosis) of keratinocytes. There is a report of EM in which herpesvirus has been implicated in the cat\textsuperscript{18}.

Lesions are often pleomorphic with an acute onset of erythematous plaques and macules that often become annular or serpiginous as they coalesce or they may appear targetoid. Progression to ulcerations is common and lesions may become variably crusted. Lesions are often generalized but are most commonly found on the ventrum, axillae, inguinal region, mucocutaneous junctions, oral cavity and pinnae. Biopsies should be obtained from areas of erythema without ulceration or crusting as an intact epidermis is needed for the diagnosis. Histologically, apoptosis with lymphocyte satellitosis is the characteristic microscopic lesion of EM.

Prognosis for EM depends on the severity of the disease and identification of underlying triggers. Use of immunosuppressive drugs in human medicine is controversial as EM is often induced by herpes simplex virus\textsuperscript{17}. In veterinary medicine, EM patients should be evaluated for underlying triggers: drugs, infection or neoplasia. Erythema multiforme minor may resolve on its own but more severe cases are often treated with immunosuppressive therapy with glucocorticoids with or without other corticosteroid sparing immunosuppressive drugs (azathioprine, cyclosporine, chlorambucil). Severe generalized mucocutaneous EM (EM major) often requires aggressive supportive care in addition to removal of underlying triggers and immunosuppressive therapy.
References

WHEN CAN THE DERMATOLOGIST CAUSE PROBLEMS FOR THE INTERNIST?

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Introduction
Management of common feline dermatological conditions frequently requires the use of drugs such as corticosteroids that have the potential to cause significant side-effects and/or complications of concurrent diseases. These notes will focus on some commonly used therapeutics and specifically address medical complications that the dermatologist should be aware of.

Corticosteroids - associated complications of glucocorticoid use
Whilst cats are commonly described to be ‘more tolerant’ of corticosteroids (glucocorticoids and sex steroids) compared to canine patients, the potential for side effects and complications clearly also exists, especially in association with high dose therapy and/or chronic treatment. The author commonly uses the glucocorticoid prednisolone to manage medical conditions, such as feline asthma, inflammatory bowel disease and IMHA; however, doses greater than 2mg/kg/day are infrequently required and tapering of doses is performed once disease remission is reached (typically using adjunctive treatment concurrently). Higher doses of glucocorticoids (GCC) are often recommended for treatment of dermatological conditions, based on clinical experience and studies reporting that feline hepatic and cutaneous tissues have approximately half the density of glucocorticoid receptors with lower binding affinity compared to dogs. However, GCC are undoubtedly one of the most overused class of drugs in veterinary medicine and there is limited evidence for very high dose regimes. Careful selection of dose, duration of treatment and required potency of GCC agent is essential to minimise side-effects; dose according to clinical response, aiming to use the lowest effective dose for the shortest period of time necessary. The use of repository agents can often be avoided by educating owners how to administer oral medications directly or by disguising tablets within foods or treats. The systemic effects and some of the potential complications of glucocorticoids are detailed in Table 1.

Diabetes mellitus
GCC have the potential to induce diabetes mellitus (DM) in cats. Feline DM is considered to be analogous to Type 2 DM in human beings, where there is reduced insulin production by pancreatic beta cells (pancreatic dysfunction) and insulin resistance. The risk of DM is therefore more likely when the cat has reduced pancreatic reserve and/or other factors antagonise the effect of insulin (e.g., obesity, GCC, progestogens). GCC antagonise the effect of insulin by several mechanisms including inducing hepatic gluconeogenesis and reducing peripheral uptake of glucose, leading to a net effect of increased blood glucose levels. Hyperglycaemia leads to pancreatic beta cell glucose toxicity, further reducing the production of insulin by the pancreas.

It is important to be aware of this potential complication in all cats prescribed GCC treatment; careful monitoring for signs of hyperglycaemia (e.g., polyuria, polydipsia due to osmotic
diuresis) is important to enable early identification of this complication. Prompt recognition of persistent hyperglycaemia will allow rapid remedial steps to be taken and may prevent the development of permanent insulin dependent DM.

1. Risk factors for DM – high dose and chronic GCC treatment, progestagen therapy, pancreatitis (acute/chronic), obesity, adrenal disease (hyperadrenocorticism, hyperprogesteronism), acromegaly; consider whether the patient is ‘at risk’ before starting GCC therapy – pay attention to even mildly elevated baseline blood glucose levels which may be consistent with a pre-diabetic state (exclude stress hyperglycaemia).
2. Diagnosis - persistent hyperglycaemia, glycosuria, raised fructosamine.
3. Management – withdrawal/dose reduction of GCC (addition of adjunctive treatment), transition to a low carbohydrate/high protein food, insulin therapy (maybe able to be withdrawn once stabilised and insulin antagonist effect has been removed), treatment of other co-morbidities.

**Cutaneous complications**
The signs and mechanism of these changes are detailed in Table 1 and are consistent with an ‘iatrogenic hyperadrenocorticoid’ state. Confirmation of this is made by identification of compatible clinical signs, history of prior GCC treatment and a flat-line cortisol response to ACTH stimulation.

**Steroid hepatopathy**
Hepatopathy is a lesser-recognised complication in cats treated with GCCs compared to dogs, in part due to the lack of a steroid-induced ALKP isoenzyme and shorter half-life of ALKP in cats. Histopathological evidence of steroid hepatopathy (characterised by glycogen accumulation in hepatocyte vacuoles ‘vacuolar hepatopathy’) was described in a study of 14 cats treated for 56 days with immunosuppressive doses of dexamethasone or prednisolone (Lowe et al, 2008). Mild elevation in hepatic enzyme activities (ALKP, ALT and GGT) may be seen.

**Infectious complications**
1. Recrudescence of feline herpes virus
2. Recrudescence of toxoplasmosis
3. Opportunistic infections (occult urinary tract bacterial infections)

**Congestive heart failure (CHF)**
CHF is a rare but recognised complication of GCC therapy in cats, even in the absence of pre-existing cardiac disease. Of the cases reported, those cats surviving the acute crisis had cardiac medications discontinued after a period of stability and following withdrawal of corticosteroids, suggesting this is a potentially reversible complication if recognised and managed appropriately. The pathophysiology of this condition is not fully understood; possible reasons for the development of congestive heart failure include an increase in circulating blood volume, vascular reactivity and possible myocardial remodelling. It is likely that the risk of development of congestive heart failure will be higher in those cats with pre-existing cardiac disease. However, identification of occult cardiomyopathy can be challenging; many cats with cardiac disease do not have heart murmurs and benign flow murmurs are commonly auscultated in...
hospitalised cats. Owners should be advised to monitor for changes in activity levels, respiratory rate and pattern when cat are receiving GCCs.

**Gastrointestinal side effects**
Inappetance, vomiting and diarrhoea may be side effects of GCC therapy; consider whether there could be a concurrent enteropathy, pancreatitis or hepatopathy that could increase the risk of these clinical signs, and only commence treatment once the cat is well hydrated (risk will be higher in scenarios where there is reduced gastrointestinal tract perfusion). Mechanisms for these effects include increased gastric acid secretion and reduced mucosal ulceration healing.

**Renal side effects**
GCC will inhibit the COX inflammatory pathway similar to NSAIDs; therefore, GCC treatment should only be commenced when the patient is well hydrated, euvaemic and normotensive. The risk of nephrotoxicity associated with COX inhibition occurs when there is hypovolaemia or hypotension and the production of prostaglandins that help maintain renal perfusion is inhibited.

**Polyuria/polydipsia**
This may occur due to the development of hyperglycaemia and an associated osmotic diuresis, however some texts describe this may develop before hyperglycaemia with an unknown aetiology (in dogs this is attributed to antagonism of ADH).

**Behavioural changes**
This is a poorly described phenomenon but anecdotally owners occasionally report marked temperament changes and/or lethargy.

**Progestogens – complications and side effects**
Progestogens such as megoestrol acetate exert similar effects to glucocorticoids and complications with therapy are comparable to those detailed above. Progestogens cause profound adrenal gland suppression. Additional potential complications include the development of cystic endometrial hyperplasia +/- pyometra, mammary hypertrophy and mammary neoplasia due to the anti-oestrogen effects of the compounds.

**Diet trial therapy - complications**
Exclusion diet trials are commonly required when investigating for food allergic dermatitis in cats. The use of modern veterinary therapeutic diets ensures that the foods are generally highly digestible and nutritionally well balanced. One possible complication is the development of hepatic lipidosis, if the trial is not well supervised. Hepatic lipidosis may be primary (unknown cause) but is more commonly a secondary process arising as a complication of concurrent disease (e.g., pancreatitis, neoplasia, gastrointestinal or endocrine disease) or starvation. The syndrome is characterised by hepatocellular lipid accumulation, intra-hepatic cholestasis and hepatic dysfunction.

A. Recognition of hepatic lipidosis
   a. Clinical signs – lethargy, inappetance, weight loss, vomiting, jaundice, weakness, neurological signs if hepatic encephalopathy has developed (ptyalism, ataxia, reduced mentation, seizures) +/- signs of concurrent disease.
b. Laboratory abnormalities – markedly increased ALKP activity, mild – moderately increased ALT activity, normal to only mild increase in GGT, hyperbilirubinaemia, hyperlipidaemia, hyperglycaemia, +/- raised ammonia and bile acids; prolonged coagulation times (PT and APTT), mild non-regenerative anaemia and electrolyte derangements.

c. Ultrasonographic features - diffusely hyperechoic hepatic parenchyma, rounded hepatic margins and hepatomegaly.

d. Definitive diagnosis – FNAB or liver biopsy for cytology or histopathology respectively; hepatocyte vacuolation, lipid-filled vacuoles; exclusion of other causes of the clinical signs and hepatopathy should be made.

B. Prevention of hepatic lipidosis during a diet trial

a. Calculate the cat’s resting energy requirement (RER) and then maintenance energy requirements (MER) in kcal:

1. **Resting energy requirement** (RER) (kcal/day)
   a. for cats < 2kgs = bodyweight (kg)^0.75 x 70
   b. for cats > 2kgs = (30 x bodyweight (kg)) + 70

2. Maintenance energy requirement (MER)
   a. for minimally active indoor adults MER = 1.0-1.2 x RER
   b. for active neutered cats MER = 1.2-1.4 x RER
   c. for active entire cats MER = 1.4-1.6 x RER

b. Ensure the owner is given *specific* instructions for the expected quantity (in grams) of prescribed food to be eaten, to meet MER/day.

c. Provide the owner with details of how to transition onto the diet
   1. Introduce the new food gradually, making the transition over 10-14 days, to maximise the chance of diet acceptance and minimise the risk of food aversion.
   2. Initially mix the foods together (e.g., combine the old and new wet or dry formulas), offer the new food alongside the current diet or offer some of the new food before the current diet ration is given.
   3. Gradually build up the proportion of the new diet over the 10-14 day period, so that the entire ration is the new diet by day 10-14. This approach improves compliance and reduces the risk of food aversion.
   4. Ensure the client contacts the clinic at any point during the transition if food intake drops; a period of starving the cat to induce acceptance of the new food is not appropriate.
   5. If the diet is not being accepted well consider either
      a. An alternative formulation (meat, paté or kibble) or completely different preparation
      b. Temporary use of an appetite stimulant (e.g. mirtazapine 1.88mg PO q24-48hrs)

C. Treatment of hepatic lipidosis

The cornerstone of treatment is nutritional support to reverse fat and protein catabolism — typically, assisted feeding via an enteral feeding tube (initially naso-oesophageal, then oesophagostomy/PEG) is necessary alongside intravenous fluid therapy to address dehydration, electrolyte and acid-base derangements. Additional supportive measures usually required include anti-emetics, anti-oxidant therapy, vitamin K1, ursodeoxycholic acid +/- agents for management of hepatic encephalopathy (lactulose
+- antimicrobials). Carnitine and thiamine may also be supplemented. Investigations for an underlying cause need to be considered, if more than simple starvation is suspected, once the cat has been stabilised.

Prognosis for recovery is generally good with rapid recognition of the condition and intensive support care.

**Doxycycline therapy – side effects and complications**

Doxycycline is generally well tolerated; however, gastrointestinal complications arise occasionally.

1. **Diarrhoea, vomiting and inappetance**
   May be overcome by dividing the dose to twice daily administration and/or administering with food. Usually develops within first few days of treatment.

2. **Oesophagitis and oesophageal strictures**
   In certain areas doxycycline is only available as a hyclate/hydrochloride tablet formulation, which if left within the oesophagus after pilling, can cause oesophageal irritation leading to oesophagitis and the development of strictures. This is a severe complication and owners must be instructed that any doxycycline hyclate preparation must be followed by a bolus of food or water (treat, butter, 2.5mls of water), to prevent this serious potential complication. Treatment of oesophagitis requires antacids (omeprazole), mucosal protectants (sucralfate), modification to a soft/slurry food consistency and opioid analgesia (buprenorphine). Oesophageal strictures are managed with balloon dilation under anaesthesia, the above drug therapeutic regime and often require placement of a PEG feeding tube until the oesophagus has recovered. Note other pills/tablets have been associated with the development of oesophagitis too (clindamycin), and it is best practice to advise owners of cats to follow any tablets or capsules with a treat or some food to ensure that the medication moves directly into the stomach lumen.

**Ciclosporine – side effects and complications**

For details regarding the use of this drug in cats, see a recent review article (Whitehouse W, Viviano K. Update in feline therapeutics: clinical use of 10 emerging therapies. *J Fel Med Surg* 2015; 17: 220-234)

Selected References

11. www.youtube.com/user/iCatCare
   Video clips providing owner instructions regarding administration of medications (all forms) and how to disguise medications within foods
Introduction
Endocrinopathies are common diseases amongst middle aged-older cats, with hyperthyroidism and diabetes mellitus being most frequently diagnosed. This lecture will cover a practical approach to the diagnosis of the most common endocrinopathies considering aetiology, case presentation and diagnostic tests.

Hyperthyroidism
Aetiology. The majority of cases of hyperthyroidism are due to benign thyroid adenomatous hyperplasia or adenomas resulting in excessive secretion of thyroid hormones (T3 and T4); only 1-3% of cases are attributed to thyroid carcinoma. In most cases reaching a diagnosis of hyperthyroidism is straightforward when there are supporting clinical signs, a palpable thyroid goitre and an elevated total T4 (TT4).

Signalment. Hyperthyroidism has been described as the most common feline endocrinopathy diagnosed in mature and older cats; the apparent prevalence in English cats over 10 years was recently reported to be 8.7%. Historically the condition was associated with cats in the senior (11-14 years) and geriatric (≥15 years) age groups, however the diagnosis is being made more frequently in younger cats (between 6-10 years of age), which may in part be explained by increased awareness and routine screening, identifying earlier disease. Domestic breeds are most commonly affected (except where the domestic cats have significant pedigree ancestry). There appears to be a degree of regional variation with some European countries reporting lower prevalence e.g. Portugal compared to the UK, USA and Australasia.

Clinical signs-Historical features. Owners may observe polyphagia, polydipsia, vomiting, diarrhoea, weight loss as well as altered behaviour including irritability, vocalisation, hyperactivity and reduced grooming. With early diagnosis, signs may be absent or very subtle such as minor weight loss or occasional vomiting. It is important to recognise that approximately 10% of cats have an apathetic form of hyperthyroidism, whereby inappetance, lethargy and weight loss may be the main presenting signs; this usually occurs when the cat has undiagnosed hyperthyroidism and a second condition e.g. congestive heart failure, pancreatitis, neoplasia.

Clinical signs-Abnormalities on physical examination. A careful examination for a thyroid goitre should be made in all cats as part of a routine physical examination; a gentle approach should be used palpating from the ventral larynx to the xiphisternum bilaterally to localise the thyroid gland(s) and assess for any enlargement. Bilateral disease occurs in ≤70% of cases. Between 3-25% of cats have more than two areas of thyroid pathology and/or ectopic tissue (which can be anywhere from the base of the tongue to the base of the heart). When a thyroid goitre cannot be palpated in the ventral cervical region but hyperthyroidism is thought likely, consider whether
the gland(s) has descended into the mediastinum, or whether examination under light sedation could be helpful.

Reduced body condition, an unkempt coat and reluctance to settle are commonly seen. Other physical abnormalities may represent effects of hyperthyroidism upon the cardiovascular system e.g. tachycardia (heart rate >180-220bpm), systolic heart murmur, arrhythmia (including gallop rhythm) and dyspnoea (if congestive heart failure develops) and/or associated systemic hypertension (e.g. weakness, ataxia, hyphaema, acute blindness, seizures). Muscle weakness is occasionally seen and may lead to a decreased ability to jump, exercise intolerance, tachypnoea and cervical ventroflexion.

Diagnostic testing
1. Routine haematology, biochemistry and urinalysis - see Table 1. Note hyperthyroidism may mask azotaemia and hence identification of concurrent chronic kidney disease; renal parameters (urea, creatinine, phosphate and urinalysis) should be repeated once euthyroidism is restored.
2. Thyroid specific diagnostic tests
3. Thyroid hormone testing - confirmation of hyperthyroidism is made by identification of a raised total T4 in 90% of cats. Radioimmunoassay or chemiluminescent enzyme immunoassay methodologies are preferred; be aware in-house ELISA tests may give discordant results. There are a few scenarios where interpretation of TT4 results may not be so simple:
   a. Unexpected elevated TT4 on a geriatric biochemistry panel
      Consider whether this could be due to an atypical presentation (‘apathetic hyperthyroidism’) or a spurious result due to laboratory error
   b. Normal TT4 despite clinical suspicion of hyperthyroidism
      Consider whether this is due to
      i. Early or mild hyperthyroidism - TT4 levels can fluctuate in and out of the normal range in early or mild cases of hyperthyroidism
      ii. Sick euthyroid syndrome - the effect of concurrent non-thyroidal disease suppressing TT4 to within reference interval; only likely to suppress mild elevations in TT4
         1. A cat with normal thyroid function and non-thyroidal illness - TT4 is likely to fall below reference interval or in lower half of the reference interval (<25nmol/l)
         2. A cat with mild hyperthyroidism and non-thyroidal illness - TT4 likely to fall in upper half of the reference interval
         3. The degree of TT4 suppression is related to severity of non-thyroidal illness
      iii. If TT4 <25nmol/l, it is unlikely that the cat is hyperthyroid, if TT4 is between 25-50nmol/l, additional testing is advisable:
      iv. Measure free T4 (fT4) (by equilibrium dialysis or chemiluminescence enzyme immunoassay)
         1. Less susceptible to effects of non-thyroidal illness
         2. 6-20% false positives
         3. fT4 should be interpreted in conjunction with TT4
4. high normal TT4 and high free T4 supports diagnosis of hyperthyroidism
   v. low to low normal TT4 (<25nmol/l) and elevated fT4 most likely non-thyroidal illness
   vi. If the cat is well alternatively re-measure TT4 and fT4 after 4-6 weeks
    vii. Treat co-morbid condition and reassess TT4/fT4 at later date

4. Additional thyroid specific tests
   These are rarely required but are included for purposes of completeness
   a. TSH levels
      i. Can be measured using canine TSH assay
      ii. Suppressed in 98% of hyperthyroid cats; should be used in conjunction with T4 and fT4 levels.
      iii. High sensitivity but poorly specific test if used alone (30% of euthyroid cats in one study had non detectable TSH levels; Peterson et al, 2015)
   b. T3 suppression test
   c. Scintigraphy
      i. Localises all hyperfunctional thyroid tissue using a radioactive tracer
      ii. Requires specialised facilities for nuclear medicine

Diabetes mellitus
Aetiology. Feline diabetes mellitus (DM) is similar to human type 2 DM arising due to pancreatic β cell dysfunction and insulin resistance. β cell dysfunction may be due to pancreatic islet amyloidosis, glucose toxicity and/or inflammation. Causes of insulin resistance include obesity, pancreatitis, concurrent endocrinopathies (hyperadrenocorticism, acromegaly, hyperprogesteronism) and certain drugs (e.g. corticosteroids, progestagens).

Signalment. Cats over 7 years of age are at greatest risk of developing DM. Male and neutered cats are most commonly affected. Burmese cats are over-represented in many studies. Obese, indoor and inactive cats are also at greater risk.

Clinical signs-Historical features. Typical signs include polyphagia, polyuria, polydipsia, lethargy and weight loss. Weakness, altered hindlimb gait, depression, collapse and anorexia are less common features with the later three signs associated with the development of diabetic ketoacidosis.

Clinical signs-Abnormalities on physical examination. May include reduced body condition, muscle atrophy, poor coat condition, mild dehydration and rarely plantigrade hindlimb stance. Vomiting, collapse and signs of shock may feature in diabetic ketoacidosis.

Diagnostic testing.

1. Routine haematology, biochemistry and urinalysis - see Table 1. Confirmation of DM is reached by the identification of a persistent hyperglycaemia (ideally fasting) and glycosuria, with compatible clinical signs. The most challenging aspect in reaching a diagnosis can be distinguishing true hyperglycaemia from stress-induced hyperglycaemia. Glucose levels can reach up to 20mmol/l due to stress alone (serum levels >10-15mmol/l may result in transient glycosuria) and affected cats may not
outwardly appear stressed. In this scenario, repeating a blood glucose a few hours later, testing a urine dipstick on a home collected urine sample 24 hours later and testing fructosamine may enable a diagnosis to be reached.

2. Fructosamine is a glycolated protein and levels reflect blood glucose concentrations over the preceding 7 days. Levels may not be elevated in recent onset DM and/or mild DM. Hyperthyroidism may reduce fructosamine levels by causing increased protein catabolism.

3. Diabetic ketosis/ketoacidosis is confirmed when there is evidence of ketones in blood (some labs measure β-OH butyrate) or in the urine (dipsticks measure acetoacetic acid) +/- metabolic acidosis.

4. Ancillary testing is recommended to search for causes of insulin resistance once a diagnosis has been made. Consider performing urine bacterial culture (signs of cystitis may not be present even with a UTI), measuring fPLI for evidence of possible pancreatitis and IGF-1 for hypersomatotropism/acromegaly.

**Hyperadrenocorticism ‘Cushing’s syndrome’**

Aetiology. Hyperadrenocorticism (HAC) is a rare feline endocrinopathy due to a functional pituitary (PD-HAC) or adrenal cortex (AD-HAC) tumour causing hypercortisolaemia; 75-80% of all cases are caused by PD-HAC (50% macroadenoma, 50% microadenoma) and 20-25% are due to AD-HAC, of which two-thirds have benign adenomas. Iatrogenic hyperadrenocorticism may develop due to exogenous topical or oral corticosteroids. Adrenal tumours secreting sex hormones or multiple hormones (e.g. aldosterone and progesterone) have occasionally been reported manifesting with similar clinical features. Development of diabetes mellitus secondary to hypercortisolaemia occurs late in the disease but is common (80-90% cases).

Signalment. HAC is most frequently diagnosed in mature and older cats (median age 10-11 years), with females slightly over-represented. There is no known breed predisposition.

Clinical signs-Historical features. Typical signs include polyphagia, polyuria, polydipsia (attributed mostly to secondary DM), lethargy and change in weight (gain or loss) and body conformation. Cutaneous changes including an unkempt coat, change in colour, alopecia and tearing skin may be described by owners. Recurrent infections may occur due to immunosuppression. Weakness due to muscle atrophy +/- peripheral neuropathy or central nervous system (CNS) abnormalities (circling, vocalisation, obtundation, blindness, incoordination) may occasionally be seen, the later due to the effect of pituitary macroadenoma expansion +/- haemorrhage.

Clinical signs-Abnormalities on physical examination. May include altered body condition (pendulous abdomen with hepatomegaly, generalised muscle atrophy) and dramatic cutaneous changes; bilateral symmetrical hair thinning or alopecia, skin atrophy and marked thinning (fragile and easily torn-*care handling*), seborrhoeic coat and bruising. Rarely, a plantigrade hindlimb stance due to peripheral neuropathic effects and/or signs referable to a space-occupying effect in the CNS are observed.
Diagnostic testing.
1. Routine haematology, biochemistry and urinalysis - see Table 1. Most of the biochemical and urine abnormalities that are documented arise due to the development of secondary diabetes mellitus.

2. Screening tests - stage 1 for diagnosis of HAC
   a. ACTH stimulation test – distinguishes iatrogenic HAC vs. spontaneous HAC. Poorer sensitivity compared to use in dogs (35-40% vs. 85%), false positives may be due to systemic illness; discuss post ACTH sample timing with laboratory. A suppressed or a flat line cortisol result is consistent with iatrogenic HAC (or sex hormone secreting adrenal neoplasia, if there is no history of exogenous corticosteroid use).
   b. Low-dose dexamethasone suppression test – requires use of 0.1mg/kg dexamethasone (10x higher dose vs. dogs) and cortisol is measured at 0,4 and 8 hours; suppression excludes HAC [suppression = 4 and 8 hour (cortisol) <50% baseline cortisol concentration or <35nmol/l].
   c. Urinary creatinine: cortisol ratio - must be tested on urine collected at home to minimise the effect of hospital stress. High sensitivity but poor specificity; values lies in a negative result making HAC unlikely.

3. Discriminatory tests – stage 2 to distinguish between PD-HAC and AD-HAC and hence determine most appropriate treatment
   a. High-dose dexamethasone suppression test - performed using 1mg/kg dexamethasone IV and cortisol is measured at 0, 4 and 8 hours. Cortisol levels do not suppress in AD-HAC, however as <50% PD-HAC cases suppress this is not a reliable discriminatory test.
   b. Endogenous plasma ACTH measurement – must be collected on EDTA and immediately chilled; discuss handling and sample requirements with laboratory. High or high normal [ACTH] expected in PD-HAC, low or low normal [ACTH] in AD-HAC.
   c. Abdominal ultrasound – bilateral symmetrical adrenal glands are expected in PD-HAC (normal – increased size); AD-HAC unilateral adrenomegaly. Useful to assess for abdominal metastatic lesions and tumour expansion into vena cava in cases of AD-HAC.
   d. Brain CT/MRI to assess for pituitary masses in PD-HAC (may not identify microadenomas).

Selected References


# Table 1. Feline endocrinopathies: summary of routine screening test abnormalities and diagnostic tests

<table>
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<tr>
<th>Disease</th>
<th>Cause</th>
<th>Endocrine disease diagnostic testing</th>
<th>Common abnormalities on screening laboratory tests</th>
<th>Ancillary tests</th>
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<td>First line diagnostic test(s)</td>
<td>Additional endocrine tests</td>
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<td>Diabetes mellitus</td>
<td>Lack of endogenous insulin</td>
<td>Persistent hyperglycaemia (including fasted sample) and glycosuria</td>
<td>Fructosamine</td>
<td>RBC, MCV; (mild); stress leukogram</td>
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<tr>
<td>Hyperadrenocorticism (HAC); PD-HAC - pituitary dependent, AD-HAC - adrenal dependent</td>
<td>Excess ACTH &amp; cortisol (PD-HAC); excess cortisol (AD-HAC)</td>
<td>ACTH stimulation test, low dose dexamethasone suppression test; endogenous ACTH; urinary creatinine: cortisol</td>
<td>Pituitary CT/MRI for PD-HAC; abdominal ultrasound</td>
<td>Mild proteinuria, ↓ specific gravity</td>
</tr>
<tr>
<td>Hyperaldosteronism ‘Conn’s syndrome’</td>
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<td>Aldosterone (serum/plasma)</td>
<td>Plasma renin activity; aldosterone: renin ratio;</td>
<td>Stress leukogram; neutrophilia alone</td>
</tr>
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<td>Acromegaly/hyper-somatotropism</td>
<td>Excess growth hormone</td>
<td>IGF-1 (serum)</td>
<td>Growth hormone (serum/plasma)</td>
<td>Mild ↑RBC</td>
</tr>
</tbody>
</table>

**Haematology**

- ↑PCV if dehydrated
- ↑glucose, ALT, ALKP
- Glycosuria +/-ketonuria; pyuria & proteinuria if concurrent UTI
- ↓PLI (for pancreatitis), urine culture (+ve bacterial culture ~11% cases), IGF-1

**Biochemistry**

- ↑ALT, ALKP, (AST), PO4;
- ↓ creatinine, ↓ K+
- ↑ALT, ALKP, (AST), cholestrol;
- ↑glucose if 2° DM
- ↑ or normal Na”; ↓ or low normal K+; azotaemia
- ↑ specific gravity if concurrent CKD
- ↑ total protein, ALT, ALKP, cholestrol, PO4;
- ↓specific gravity if 2° DM
- Mild proteinuria;
- Glycosuria if 2° DM

**Urinalysis**

- Mild proteinuria, ↓ specific gravity
- Systolic blood pressure, urine culture (+ve bacterial culture 12% cases), assessment renal function once euthyroid
- Systolic blood pressure
- Urine culture
# Table 2. Major systemic effects of glucocorticoids and potential adverse effects

<table>
<thead>
<tr>
<th>System</th>
<th>Possible changes induced by glucocorticoids</th>
<th>Potential effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic</td>
<td>Gluconeogenesis</td>
<td>Hyperglycaemia→ diabetes mellitus, osmotic diuresis (PU/PD); vacuolar hepatopathy</td>
</tr>
<tr>
<td></td>
<td>Ketogenesis</td>
<td>Hyperglycaemia, diabetes mellitus</td>
</tr>
<tr>
<td></td>
<td>Glycogen synthesis</td>
<td>Hyperglycaemia, diabetes mellitus</td>
</tr>
<tr>
<td></td>
<td>Antagonism effect of insulin in peripheral tissue</td>
<td>Muscle atrophy, generalised weakness, reduced exercise tolerance, skin thinning, pendulous abdomen, reduced wound healing</td>
</tr>
<tr>
<td></td>
<td>Decreased release of insulin from pancreatic beta cells</td>
<td>Increased intra-abdominal fat, vacuolar hepatopathy</td>
</tr>
<tr>
<td></td>
<td>Protein catabolism</td>
<td>Soft tissue mineralisation, calcinosis cutis</td>
</tr>
<tr>
<td></td>
<td>Lipolysis, fat redistribution</td>
<td>Biochemical effect shown with immunosuppressive dose prednisone but effects on thyroid axis less well characterised in cats vs. dogs</td>
</tr>
<tr>
<td></td>
<td>Increased PTH, bone turnover, calcium mobilisation and excretion</td>
<td></td>
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<tr>
<td></td>
<td>Reduced serum total T4 (altered plasma protein binding)</td>
<td></td>
</tr>
<tr>
<td>Inflammation and the immune system</td>
<td>Decreased neutrophil margination, reduced neutrophil migration to sites of inflammation, lymphocyte redistribution to extravascular sites</td>
<td>Stress leukogram: neutrophilia, lymphopenia, eosinopenia, monocyteosis (much &lt;common cats than in dogs)</td>
</tr>
<tr>
<td></td>
<td>Reduced macrophage phagocytosis and expression of anti-inflammatory cytokines (CK), decreased prostaglandin production</td>
<td>Decreased antigen uptake, reduced inflammatory response</td>
</tr>
<tr>
<td></td>
<td>Decreased T lymphocyte activation, proliferation and CK production</td>
<td>Reduced cell mediated immune response</td>
</tr>
<tr>
<td></td>
<td>Decreased antibody synthesis (high dose long term treatment only)</td>
<td>Reduced humoral immune response</td>
</tr>
<tr>
<td></td>
<td>Induction lipocortin-1</td>
<td>Decreased margination &amp; migration neutrophils; reduced production inflammatory eicosanoids</td>
</tr>
<tr>
<td>Dermatological</td>
<td>Atrophic effects-suppression keratinocyte and dermal fibroblast proliferation, suppression fibroblast derived proteins (including collagen), decreased epidermal lipids and transepidermal water</td>
<td>Epidermal and dermal thinning, follicular atrophy, easy bruising, reduced wound healing; thin and fragile skin, pinnal tip curling, alopecia, dry coat</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Exacerbation of cardiac disease (occult/non-occult)/induction cardiomyopathy</td>
<td>Corticosteroid associated congestive heart failure</td>
</tr>
<tr>
<td>Nervous system</td>
<td>Appetite stimulation, reduced neuronal excitation</td>
<td>Polyphagia, lethargy, behavioural abnormalities</td>
</tr>
</tbody>
</table>
APPROACH TO SCALING AND CRUSTING AND MANAGEMENT OF
DERMATOPHYTES AND BACTERIA AND DERMATOPHILUS IN HORSES

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**Dermatophytes and Malassezia**

Dermatophyte infections, like pyoderma, can be variably pruritic. The most common equine dermatophyte species isolated from horses are *Trichophyton equinum, M. canis, M. equinum, M. gypseum, T. mentagrophytes, T. bullosum and T. verrucosum*. Tack (bridles, halters, saddle blankets) often act as fomites. The lesions usually appear first on the axillary/girth area and may spread over the trunk, rump, neck, head and limbs. Initial lesions may be urticarial in nature progressing to multiple focal sharply demarcated scaling and crusting areas. Lesions may be superficial or deep. Superficial infections are more common and are manifested by the development of thick crusts, or more generally a diffuse moth-eaten appearance with desquamation and alopecia. Less commonly, deeper structures are infected through the hair follicles causing small foci of inflammation and suppuration. A small crust forms over the follicle and the hair is lost but extensive alopecia and crust formation do not occur.

Diagnosis is by identifying microconidia along hair shafts on acetate strip evaluation; Polymerase Chain Reaction (PCR) now available at selected laboratories for a few species; fungal culture; and/or cutaneous biopsies (NOTE: *Trichophyton* species may also cause acantholysis, mimicking pemphigus on histopathology – ensure a fungal stain is pursued in any case of suspected pemphigus). Clear packing tape is used to collect samples from the periphery of affected sites. The tape strip is then suspended at the end of a glass slide and stained using the eosin and methylene blue Diff Quik dips (#2 and #3; avoid dipping in the methanol #1 as it may dissolve the glue of the tape). After staining is complete, remove the tape from the end of the slide and readjust the strip so that it lays over the entire slide for microscopic evaluation.

Hair is the specimen most commonly collected for the isolation of dermatophytes for PCR and fungal cultures. Using forceps, hairs should be selected that appear stubbed and broken, especially at the advancing periphery of an active, non-medicated lesion. In addition, surface keratin may be gathered by forceps or skin scrapings from similar areas and inoculated onto the culture medium. The hair and surface keratin of large animals have large numbers of saprophytic fungi and bacteria. Hence, it is recommended to cleanse the skin prior to taking samples for culture. This may be done by gently cleansing the area to be sampled with soap and water or alcohol, allowing it to air dry before acquiring samples. Samples for PCR or fungal culture are then submitted to the laboratory in a sterile urine container.

If performing cultures in the clinic, Sabouraud’s dextrose agar has been used traditionally in veterinary mycology for isolation of fungi; however, other media are available with bacterial and fungal inhibitors, such as Dermatophyte Test Medium (DTM). DTM is essentially Sabouraud’s dextrose agar containing cycloheximide, gentamicin, and chlortetracycline as antifungal and antibacterial agents and to which the pH indicator phenol red has been added. Dermatophytes utilize protein in the medium first, with alkaline metabolites turning the medium red. Most other
fungi utilize carbohydrate first, giving off acid metabolites, which do not produce a red color change. These saprophytic fungi will later use the protein in the medium, resulting in a red color change. Consequently, DTM cultures should be examined daily for the first fourteen days. Some *Aspergillus* species and others cause a red color change in DTM, so microscopic examination is essential to avoid an erroneous presumptive diagnosis. It has been recommended that one to two drops of a sterile injectable B complex vitamin preparation be added to culture plates when culturing horses, as one strain of *T. equinum* (*T. equinum* var. *equinum*) has a unique niacin requirement. Skin scrapings and hair should be inoculated onto Sabouraud’s dextrose agar and/or DTM and incubated at 30°C with 30% humidity. A pan of water in the incubator will usually provide enough humidity. Cultures should be checked every day for growth. DTM may be incubated for 21 days, but cultures on Sabouraud’s agar should be allowed 30 days to develop. The author has usually used split culture plates with DTM on one side and rapid sporulation media on the other, with a well of water in the center. It is routinely incubated at room temperature in a dark cupboard to stimulate macroconidia formation. *T. verrucosum* has been reported not to grow on DTM.

Topical treatment alone is often curative. Lime sulfur (Sulfurated Lime®, Dechra) diluted 1 cup to 1 gallon of water, or bleach 1:10 with water, are both effective, but odiferous and can cause coat colour changes. Accelerated hydrogen peroxide (Pure Oxygen Shampoo®, Ogena) is a surfactant potentiated hydrogen peroxide that exerts its efficacy at lower concentrations than the parent compound and is more stable with fewer side effects, including no coat colour changes. Miconazole 2% or ketoconazole 1% veterinary shampoos are becoming more widely used and may be as effective. Terbinafine 1% can also be used as a spray-on topical therapy. An enilconazole 0.2% rinse (Imaverol®, Elanco) is highly effective. Dilutions of 1:50 are recommended as a leave-on rinse weekly for 6-8 weeks or until two negative fungal cultures have been obtained at 2-4 week intervals. All in-contact horses should also be treated to eliminate recontamination.

Systemic treatment is occasionally needed. Griseofulvin’s efficacy in horses (as well as an effective dose) has not been determined conclusively. However, a dosage of 100 mg/kg daily for 7-10 days has been advocated. Griseofulvin is a teratogen and should not be used in pregnant mares. Alternatively, 20% sodium iodide (NaI) may be given IV (250 ml/500 kg horse every 7 days, 1 to 2 times). This also is contraindicated in pregnant mares as it may cause abortion or congenital hypothyroidism. While medications such as itraconazole and fluconazole have been used to treat horses with systemic mycotic infections, there have not been any studies on their effectiveness in dermatophytosis. However, their safety record in horses, in the face of the doses used (5 mg/kg q 24h), are encouraging. Lastly, vaccination against *T. equinum* may reduce the incidence of new infections and protect a high percentage (> 80%) of vaccinates from infection. This data is based on results with an inactivated vaccine containing both conidia and mycelial elements.

The exact species of *Malassezia* growing on horses’ skin is just beginning to be investigated. In one study, the *Malassezia* species isolated were identified as *M. furfur, M. slooffiae, M. obtusa, M. globosa, M. equine, M. pachydermatis* and *M. restricta*. The author has examined several mares with a *Malassezia* infection between their mammary glands and geldings/stallions with
prepucial dermatitis that were intensely pruritic. The mares and geldings/stallions rubbed their tail and ventral abdomen. Physical examination showed a dry, greasy-to-the-touch exudate. Cytology of the exudate showed numerous yeast organisms, which were identified on cytology as *Malassezia* species. Treatment with a topical 2% miconazole/chlorhexidine shampoo was curative. In some cases, oral fluconazole (5mg/kg/day) was necessary to address the yeast overgrowth. Remember to always correlate your clinical findings with your cytologic results before initiating treatment as even healthy non-pruritic mares have been identified with large numbers of yeasts in the intra-mammary area.

**Bacterial Skin Infections**

Bacterial folliculitis (superficial pyoderma) is usually caused by a coagulase positive *Staphylococcus* species. Both *S. aureus* and *S. intermedius* have been isolated. In one study, *S. aureus* accounted for twice as many isolates as *S. intermedius*; the same study isolated some strains of *S. hyicus* as well. Interestingly, in another study, lysozymes from equine neutrophils were only slightly bactericidal for *S. aureus*. Many isolates are resistant to penicillin G. Occurrence of pyoderma has been linked to poor nutrition and husbandry in some cases. Recent culture identification of bacterial infections in horses would tend to suggest that *S. intermedius* is more correctly identified as *S. pseudintermedius*, and that *S. delphini* may be the most common staphylococcus species isolated from horses.

Clinical signs of staphylococcal pyoderma are most often crusts, usually in a circular pattern suggestive of dermatophytosis (this may be the reason that equine pyoderma is under-diagnosed), epidermal collarettes (circular skin lesions with an exfoliative border as seen in dogs with superficial pyoderma) or encrusted papules similar to the miliary dermatitis reaction pattern in cats. These infections tend to be variable in their intensity of pruritus. Histology usually shows folliculitis and/or furunculosis, but bacterial colonies are not always seen. A truncal form of bacterial folliculitis (contagious acne, contagious pustular dermatitis, Canadian horsepox) is often associated with poor grooming, trauma from tack and saddle, warm wet weather and heavy work. It is painful and interferes with working and riding. It is usually caused by a coagulase positive *Staphylococcus* species but may also be caused by *Corynebacterium pseudotuberculosis*. This organism is more commonly identified as a cause of deep pyoderma. In horses, Corynebacterium folliculitis often develops in the saddle and lumbar region, particularly in the summer. The affected area initially may be swollen and very sensitive; this is followed by formation of follicular papules and pustules. These may become confluent or rupture, forming plaques and crusts. Deep pyoderma followed by ulceration may develop over large areas of the body, especially on the neck, sides of the thorax, inner surface of the thighs or on the prepuce.

Pastern dermatitis is often associated with a primary or more often secondary bacterial infection (pastern folliculitis). Again, the causative agent is usually a coagulase positive *Staphylococcus* species. As with most “primary pyodermas”, the mechanism(s) whereby the organism gains its foothold is unknown (not contagion, not poor sanitary conditions). The lesions are usually limited to the posterior aspect of the pastern and fetlock regions; one or more limbs may be involved. The initial lesions consist of papules and pustules. If left untreated, the lesions coalesce and may produce large areas of ulceration and suppuration, which may be quite painful. The disease is usually not associated with systemic signs and the general health of the horse is not affected.
A relatively uncommon nodular disease termed ‘botryomycosis’ mimics actinomycosis or a deep fungal infection but is most often caused by *Staphylococcus* species in the horse. These may require surgical excision as well as long-term antibiotics.

**Public Health Considerations – *Staphylococcus* spp**

Methicillin-resistant, coagulase-negative and -positive staphylococcal species have been identified throughout the globe and must be considered a potential threat to horses, owners and veterinarians. Methicillin-resistant *Staphylococcus* aureus (MRSA) was isolated from both humans (13%) and horses (4.7%) on horse farms in Canada and New York state. In looking at horses admitted to a university teaching hospital (Ontario Veterinary College), MRSA was isolated from 120 (5.3%) of 2,283 horses. Of these 120 horses, 50.8% were positive at the time of admission, and clinical infections attributable to MRSA were present or developed in 14 horses. Horses colonized at admission were more likely to develop clinical MRSA infection. Administration of ceftiofur or aminoglycosides during hospitalization was the only risk factor associated with nosocomial MRSA colonization.

Of most concern is the finding of humans reporting skin lesions following contact with a community MRSA-positive affected foal, despite short-term contact and standard protective barriers. The isolates from the foal were indistinguishable from the ones from the humans. In a recent study, MRSA was isolated from 15.6% of veterinarians or technicians in large animal practice, as opposed to only 4.4 % for small animal personnel; large animal practice was the only statistically significant variable associated with colonization of MRSA. Active screening and strict implementation of infection control protocols can help to eliminate MRSA without systemic antimicrobial treatment. (Table 1) MRSA strains common to horses and those individuals who work with them (e.g. USA 500), is no more robust in attaching to equine keratinocytes than other strains. Other strains may prove to be more resilient such as the sequence type 398 (ST398) MRSA, which has become the most common MRSA clone isolated from horses in Europe and has been involved in outbreaks in equine hospital facilities in several countries.

Ideally, antibiotic selection should be based on culture and sensitivity findings. Empirically, the antibiotic used for many bacterial skin infections in the horse is trimethoprim sulfa per os (15-30 mg/kg q12h for 2-6 weeks, longer for deep infections). Interestingly, dosing intervals for *intravenous* administration of trimethoprim-sulfamethoxazole in horses may not be appropriate for use in donkeys or mules. Donkeys eliminate the drugs rapidly, compared with horses. In cases of *Staphylococcus* spp. resistance to TMS, enrofloxacin may be used. The dose is 7.5 mg/kg PO once daily or 5 mg/kg IV once daily. Use of enrofloxacin in young horses (less than 2 years old) should be avoided, due to concerns of damage to the articular cartilage. The oral gel formulation of enrofloxacin 100mg/ml has good clinical efficacy for infections in several organs; however, almost one-third of the horses may develop diarrhea and 10% may erupt with oral lesions. Always follow oral enrofloxacin gel administration with a tap water rinse to minimize risk of adverse reactions. Ceftiofur sodium (Naxcel®, Zoetis) at 2.2 mg/kg intravenously or intramuscularly every 12 hours, or ceftiofur crystalline free acid (Excede®, Zoetis) at 6.6 mg/kg intramuscularly every 4 days should be effective in the absence of methicillin resistance. Cefpodoxime proxetil (Simplicef®) can also be used in horses for susceptible bacteria at
10mg/kg every 6-12 hours with a lower breakpoint than most cephalosporins (<2 μg/ml versus 8 μg/ml). If susceptible based on sensitivity results, doxycycline (10 mg/kg BID per os) or minocycline (4 mg/kg BID) may also be considered for treatment. Vancomycin (7.5 mg/kg q8h given IV over 30 minutes) has been used, alone or in combination with an aminoglycoside, to treat MRSA and enterococcal infections. The antibiotic, alone or in combination with an aminoglycoside, is safe and effective. Because of the problems with emerging resistance, vancomycin use in horses should be limited to cases in which culture and susceptibility indicate effectiveness and no reasonable alternative treatment is available.

For localized lesions, mupirocin ointment (Muricin®, Bactroban®) or silver sulfadiazine (Silvadene® or Flamazine®) cream may be effective. Shampoo ingredients including benzoyl peroxide, accelerated hydrogen peroxide (Pure Oxygen Ultra Shampoo®), ethyl lactate, miconazole or chlorhexidine are helpful. Medical grade honey has shown activity against the more common surface bacteria including MRSA at concentrations of 16% or greater.

One aspect of diagnosis that is often overlooked is determination of the underlying cause. Staphylococcal folliculitis is almost always a secondary problem. Efforts to identify health or management factors that predispose to infection are critical to a successful outcome. This approach may involve combinations of diagnostic testing to rule-out primary etiologies such as Cushing’s syndrome or allergies; environmental assessment including stalls and turnout areas; and evaluation of management practices including blanket, tack cleanliness and fit, and bathing practices.

**Dermatophilosis**

*Dermatophilus congolensis* is an actinomycete bacterium that typically only manifest in dermatitis when three conditions are present simultaneously: a carrier animal, moisture and skin abrasions. Chronically affected animals are the primary source of infection; however, they only become a serious source of infection when the lesions are moistened, which results in the release of zoospores, the infective stage of the organism. Mechanical transmission of the disease occurs by both biting and nonbiting flies, and possibly fomites. Because normal healthy skin is quite impervious to infection with *D. congolensis*, some predisposing factor that results in decreased resistance of the skin is necessary for infection to occur, prolonged wetting of the skin being one of the most important.

The disease is usually seen during the fall and winter months, with the dorsal surface of the horse most commonly affected. Occasionally, the lesions involve the lower extremities when animals are kept in wet pastures ("dew poisoning"), or if horses are left in the stall while the stall is cleaned with high-pressure water hoses. In the early stages of the disease, the lesions can be palpated more readily than they can be seen. Removing the crusts and attached hair exposes a pink, moist skin surface, with both the removed hair and the exposed skin assuming the shape of a “paintbrush”. The under surface of the crusts are usually concave with the roots of the hairs protruding.

Diagnosis is made by demonstrating the “railroad track” cocci on impression smears. A portion of one of the crusts should be minced and mixed with a few drops of sterile water on a glass slide, stained and examined microscopically. Alternatively, bacterial culture or histopathology
may be utilized for diagnosis. A thick crust composed of alternating layers of parakeratotic stratum corneum, dried serum, and degenerating neutrophils is the most characteristic histopathologic change. A superficial folliculitis may be a prominent feature of the disease. The branching, filamentous organisms can be observed in the stained sections with crusts and follicles.

Treatment involves housing the affected individual in a dry environment, removal of crusts from the skin (with care, as these may be painful), washing lesional areas with accelerated hydrogen peroxide, chlorhexidine or lime sulfur, and administration of systemic antibiotics (penicillin: 22,000 mg/kg procaine pen G intramuscularly twice daily for 7 days or trimethoprim sulfa orally until 7 days past clinical resolution). As the crusts are an important contagion, they should be disposed of, rather than simply brushed on to the ground.

Table 1. Measures for the control of MRSA on farms

<table>
<thead>
<tr>
<th>Individual Horse Measures</th>
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<tr>
<td>1) Isolate infected horses if possible</td>
</tr>
<tr>
<td>2) Use dedicated water buckets, feed bowls, brushes, and other items</td>
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<tr>
<td>3) Decontaminate before if using on other horses or discard after use</td>
</tr>
<tr>
<td>4) Use personal protective equipment (dedicated coveralls and gloves) when handling infected horses or entering their stall</td>
</tr>
<tr>
<td>5) Wash hands or use an alcohol-based hand sanitizer after contact with the horse or its environment, including after glove removal</td>
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<tr>
<td>6) Clean and disinfect the stall after resolution of disease</td>
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<tr>
<td>7) Turnout only in a dedicated pasture or paddock</td>
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<table>
<thead>
<tr>
<th>Group Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Limit antimicrobial use</td>
</tr>
<tr>
<td>2) Separate horses into different risk groups and limit cross-contact (direct and indirect)</td>
</tr>
<tr>
<td>3) Have a good facility infection control program and preventive medicine program to reduce the risk of opportunistic infections</td>
</tr>
<tr>
<td>4) Quarantine new arrivals</td>
</tr>
<tr>
<td>5) Ensure that culture and susceptibility testing is performed on horses that develop opportunistic infections</td>
</tr>
<tr>
<td>6) Ensure good general hygiene practices by farm personnel and other individuals that have contact with horses (e.g., farriers, veterinarians)</td>
</tr>
</tbody>
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Selected References


EQUINE PASTERN DERMATITIS

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Introduction
The pastern area is defined as the part of the equine leg between the fetlock and the top of the hoof.¹ The so called pastern dermatitis is just an umbrella term of any kind of dermatitis or dermatoses affecting this region and does not reflect a specific diagnosis. Many of the dermatological entities that affect this body part can more or less overlap in clinical presentation, making diagnostic work challenging. Furthermore, as various conditions in this area look similar, it is understandable that lay people assume that “pastern dermatitis” is one disease and that there should be one “best ointment” with ability to cure. As dermatitis affecting the pastern area is very common worldwide, the market is filled with prescription-free ointments sold with the claim of being the “number 1” treatment for pastern dermatitis, unfortunately and understandingly, none being universally effective. Horse owners have though often tried a high number of those remedies themselves, before consulting a veterinarian.

Prevalence studies are largely lacking, but although the condition is common in any adult horse, pastern dermatitis is generally considered being more common in draft horses and associated with heavy feathered legs.²³ Of 974 Franches-Montagnes or Freiberger horses, 15.2% had pastern dermatitis⁴, whereas in a German study of 917 coldblood horses >2.5 years of age, 47-98% were affected.⁵ The study further concluded that all four legs or both hind legs were affected most commonly and that occurrence was dependent of age. Pigmented pastern skin seems to be affected less frequently as compared to unpigmented areas, which was demonstrated by legs with white markings having a 2.6 times higher prevalence of pastern dermatitis in the study on Franches-Montagnes horses.⁴

Clinically, pastern dermatitis often presents as erythema, scaling, fissuring and variable sensitivity to the touch or even severe pain. Alopecia, exudation, erosions, oedema and crusting (often adherent and sometimes impressively thick) develop later in the course of the progression. Lymphangitis with fever and extreme swelling of the limb can occur. Depending of the etiology, also other clinical signs (pruritus, thick folding of the skin, verrucous projections, nodules and ulcerations) might develop.²³ Chronic inflammation can cause severe tissue changes that can become irreversible and impair the gait.

Although pastern dermatitis due to different etiologies can present similarly, there are still some clues that can aid in the work up and thereby aid to choose the most relevant diagnostic tests to avoid running all available tests on each case immediately. Any of the conditions causing pastern dermatitis can, and often will, become secondarily infected,²³ which further drives the inflammation, thus cytology always need to be performed.
Crusts and scaling without pruritus

*Bacterial infection/pyoderma* is very common and can be seen as secondary either to environmental or iatrogenic skin barrier defects or any other dermatosis/dermatitis affecting the leg. Care should be taken to explain to horse owners the importance of not damaging the skin barrier. The skin in the pastern area and any coverings including boots should be kept clean. Maceration of the skin by prolonged wetting by standing in moist or excessive rinsing of the legs should be avoided. Shaving the legs can create micro trauma thus being a risk factor of infection, but clipping heavy feathers helps to reduce skin moisture retention.

Various species of *Staphylococcus* are often implicated in the pathogenesis of pastern pyoderma, with *S. aureus*, *S. hyicus* and *S. delphini* being the most common. It is important to bear in mind that methicillin resistant *S. aureus* (MRSA) have been cultured from horses on several occasions, and that spread from horse to human can occur. Co-infection, such as with *Staphylococcus* spp. and *Streptococcus* spp., among those the β-hemolytic *Str. dysgalactiae*, is not rare, but also other bacteria (*E.coli*, *Enterobacter* and others) can be involved. Cytology is preferably taken by first removing a crust and sampling the underlying skin. Eroded, moist lesions should be avoided, as these are often contaminated and may not be representative of the infection. The surface of the skin can first be cleansed with alcohol, and then scraped with a sterile, dull scalpel blade to collect material. If a culture is to be taken, this can be done with a culture swab after the same procedure, or by taking a 2mm punch biopsy after surgically scrubbing the area.

*Dermatophilus congolensis* can cause outbreaks of pastern dermatitis, sometimes affecting several horses in a group. To become infective, *D. congolensis* needs moisture and an impaired skin barrier. Infections of unpigmented skin can induce a more severe, inflammatory reaction which might reflect a photodynamic reaction. The microorganism is extremely long-lived in crusts and acts as a source of infection. Protective immunity is not achieved after infection; re-infections occur and chronically infected cases exist. Dermatophilosis is contagious and can cause disease also in other animals, such as cattle and sheep, and is a potential zoonosis. In the acute phase, when crusts with tufted hairs on top and greenish pus on underside is seen, cytology is very rewarding and reveals thick, branching rows of cocci (“railroad tracks”). In later stages, crusts can be moistened with saline and “chopped up” on a glass slide before staining for cytology. With histopathology of skin biopsies the microorganism can be demonstrated in palisading crusts, but also in hair follicles and on the skin surface. Bacterial culture is not always rewarding, but RT-PCR technique seems to be useful.

*Dermatophytosis* is rarely restricted only to the pastern area, but can cause varying degree of scaling, crusting and alopecia. As dermatophytosis is contagious and a zoonosis, lesions are often detected in several animals in a group and sometimes also on in contact humans. With direct microscopy of hairs (trichogram), arthrospores in and along swollen, pale hair shafts might be detected. Real-Time PCR technique is today available to detect *Trichophyton* and *Microsporum* in hairs, scales and crusts and a result is available within a couple of days, but a culture is needed to discriminate between the various species. A fungal culture takes longer time (up to 2.5 weeks), but allows identification of species.
Contact reactions, allergic or just irritant, can cause erythema, scaling, crusting, alopecia and even erosions and ulcerations. Terpinene (in tea tree oil) can and chlorhexidine are examples of substances that can act as a hapten,\textsuperscript{11} inducing contact allergic reaction after sensitization.

**Crusts and scaling with pruritus**

If crusting and scaling dermatitis with pruritus occurs in during the colder months, *Chorioptic mange* is highly likely. Spontaneous improvement of the condition is commonly seen during the warmer months. Horses with heavy feathering of the lower limbs are predisposed to the mite infection. The condition is contagious, but short-haired horses can exhibit milder, sometimes no clinical signs, but can serve as a source of reinfection. Furthermore, the mite can survive several weeks in the environment. The mites are very motile and live superficially. Scraping with mineral oil on the scalpel blade, after clipping a small area, usually reveals higher numbers of mites as compared to tape preparations or scraping or brushing without clipping or the use of oil. Trombiculosis usually affects pastured hoses in the late summer/early fall. The larval stage of the free living “harvest mite” *Trombicula automnalis*, feeds on the blood of any warm-blooded animal for about 72 hours before dropping off the host to mature into nonpathogenic nymphs and adults. The bites can induce pruritic papules and crusting of the area, and the pruritus can in some horses be intense. The six-legged larva is visible macroscopically, as a small orange-red dot. With clear acetate tape it is easy to collect suspected material to be scrutinized under a magnifying lens or under the microscope for identification of the long-legged mite. Inflammation and pruritus sometimes need to be addressed by topical corticosteroids and topical miticidal products can be needed during the mite active period.

**Only unpigmented skin**

If only unpigmented skin is affected and lesions respect the border to pigmented, skin *photosensitization* should be suspected. If a photodynamic agent reaches the skin, either via circulation or percutaneously, and meets UV-radiation, the photodynamic agent becomes activated and forms reactive oxygen (peroxides, free radicals) which damage cell components in the area. As a result erythema, swelling, erosions, ulceration, necrosis, crusting, exudation and pain occur. Pigment in the skin prevents activation of the photodynamic agent, as the UV radiation is absorbed by the pigment. Distribution of a photodynamic agent via circulation can occur after ingestion of plants containing photodynamic agents [such as hypericin in St. John’s wort, and alsike clover (*Trifolium* spp.)], or by distribution of drugs with photodynamic properties (such as phenothiazine, tetracycline, and sulphonamides) or by the horse having a liver disease causing impaired elimination of phyloerythrin. Phyloerythrin is a metabolite of chlorophyll with photodynamic properties. By contact, tar, the plants *Heracleum mantegazzianum* (Giant Hogweed) and *Pastinaca sativa* var *sylvaticus* (wild parsnip) and possibly also clover can induce photosensitization, as can PABA (para-aminobenzoic acid) which still can be found in some sunscreens, and the antiseptic hexachlorophene.

*Vasculitis* (photo-aggravated vasculitis, leukocytoclastic vasculitis) mainly affects unpigmented areas. The aetiopathogenesis is as yet unclear. The condition is often painful and clinically the lesions more commonly are seen on lateral and/or medial aspects of the legs, less commonly in the caudal pastern area. Erythema, erosions, ulcerations, exudation and crusting, often with a circular, well circumscribed formation can be seen, and the leg can be edematous and thick. Affected pigmented skin can appear more alopecic and scaly.\textsuperscript{12} The diagnosis is made by
histopathological examination of deep biopsies, taken after any secondary pyoderma has been controlled.

Vasculitis can also be seen secondarily to a huge variety of conditions (such as infections, drug reactions, neoplastic diseases) or be idiopathic and induce lesions also in pigmented skin and skin in other areas of the body.

**Nodules/Proliferative lesions**
Chronic pastern dermatitis can lead to proliferative tissue changes that can become irreversible. Severe lichenification and fibrosis can occur and fibroblastic and epidermal proliferation creating multinodular, grapelike projections can develop (verrucous hyperplasia). Apart from trying to identify and control perpetuating factors (such as secondary microbial infections, Chorioptic mite infestation, contact reactions, factors inducing excessive moist/maceration of the skin, inflammation) surgical intervention can be needed and has been described successful in one affected draught horse.13

**Chronic progressive lymphedema** (CPL) is a genetically predisposed condition in which a defect in, or break-down of, elastin results in lymph vessels congestion and poor skin support. The disease is described in heavy draft horses (Shire, Clydesdale, Belgian, Ardenner, Tinker). Clinical signs can be seen in young adults, and hind limbs are often earlier and more severely affected compared to front legs. There is swelling of the tissue, creating skin folding or nodular swellings. Later in the course of the disease, erosions, ulcerations and crusts develop, and the leg can become impressively enlarged up to the tarsi/carpi level, impairing movements and gait. In the deep skin folds secondary microbial infections, mite infestation or even maggots can further increase the inflammatory reaction and worsen the condition. The disease resembles human chronic lymphedema. On deep skin biopsies, best taken as incisional biopsies, dilated lymph vessels with thickened vessel walls, dermal fibrosis and neovascularization are indicative of the disease. If special stain for elastin (such as Verhoeff’s stain) is used, abnormal elastin in the dermis and around lymph vessels can be seen. There is no cure for this progressive disease which, with time, can become a reason for euthanasia. Apart from addressing secondary, complicating factors, manual lymph drainage and compressing bandages has though been shown to improve the condition.14 Corticosteroids (prednisolone) can, especially if instituted early in the course of the disease, be helpful. In Canada low-dose dexamethasone in combination with a diuretic agent (trichlormethiazide) has been used for palliative treatment.

Furthermore, nodules of infectious or neoplastic origin can develop in the pastern area. Sarcoids, fibroblastic tumors associated with variants of bovine papilloma virus (BPV) type 1&2, often develop in sites of previous trauma, and can affect the pastern area.

**Alopecia/ Scaling**
When the clinical presentation is dominated by localized or diffuse alopecia and scaling, folliculitis due to dermatophytosis or bacterial infection (such as *Staphylococcus* spp. or dermatophilosis) needs to be considered. With bacterial folliculitis typically papules, erythema, crusting and exudation will be seen, but in the healing phase only alopecia and scaling is seen. Also vasculitis affecting pigmented limbs can present mainly with diffuse alopecia, edema and scaling.
A localized, lymphohistiocytic, granulomatous dermatitis with giant cells, histologically resembling the multisystemic disease sarcoidosis can affect regional areas of a limb. The initiating factor triggering the immune response is largely unknown. Clinically regional, diffuse alopecia, scaling, crusting, thickening of the skin and limb edema is seen. The lesional area is often well demarcated and can persist over years, and if lesions involve coronary bands also hoof horn production can be affected. Response to corticosteroids systemically, or sometimes topically, as well as topical tacrolimus has been described and often need to be maintained to prevent relapses.15

**Coronary band**

In rare cases, lower limb lesions are restricted to coronary bands with or without involvement of ergots and chestnuts. Coronary band dystrophy, a condition of unknown etiology where the coronary bands are scaly and crusting, affects all four legs with an onset in young horses. Hoof horn can become rough, with a non-even surface. Horses with multisystemic epitheliotropic eosinophilic dermatitis and stomatitis (MEEDS) can have coronary, ergot and chestnut scaling, crusting, erosions and ulceration. Oral lesions are often seen already early in the disease. In hepatocutaneous syndrome, coronary band erosions, ulceration and crusting is associated with severe liver disease. Pemphigus foliaceus, systemic granulomatous/histiocytic disease (sarcoidosis), coronary band pyoderma and vasculitis are examples on other conditions that may involve the coronary bands only or together with lesions elsewhere.

References

Introduction
Horses suffer from many different types of dermatitis. These vary from the basic and well understood dermatoses such as dermatophytosis in which a clear diagnosis can be made and for which a relatively simple evidence based treatment is available to much more complex disorders that occur only rarely and for which little evidence is available. Some of the latter are highly problematic in the diagnosis and management.

Pemphigus foliaceus, pemphigus vulgaris and bullous pemphigoid are rare auto-immune (skin) disorders affecting man and animals, including the horse. This lecture will focus on these disorders in the horse and their differential diagnoses.

Pemphigus complex
The term ‘pemphigus’ is derived from the Greek ‘pemphix’ meaning ‘blister.’ The word ‘foliaceus’ comes from the Latin ‘folium’ meaning leaf. The word ‘vulgaris’ comes from Latin and means common. In the horse this makes things a bit confusing as pemphigus vulgaris is in humans the most common pemphigus form while in the horse pemphigus foliaceus is seen rarely but much more frequently than pemphigus vulgaris (and bullous pemphigoid).

Pemphigus foliaceus and pemphigus vulgaris are classified under the ‘pemphigus complex’. Bullous pemphigoid is currently classified under the ‘sub-epidermal bullous dermatoses’, and contrary to the dog and cat, this is in the horse the only condition falling into that group of conditions. Further, in the horse, pemphigus vulgaris and bullous pemphigoid are clinically very similar entities with a largely identical therapy and the same very poor prognosis. Both entities can only be differentiated through a histological biopsy.

Clinically, pemphigus foliaceus, pemphigus vulgaris and bullous pemphigoid have different appearances varying from some crusting, scaling and tufted papules to very thick annular crusts, areas of alopecia and variable degrees of oozing of proteinaceous fluid (exudate). Histologically, pemphigus foliaceus and pemphigus vulgaris are characterised by intraepidermal acantholysis leading to pustule or vesicle formation. In cases of pemphigus foliaceus the mucous membranes are not involved, but in cases of pemphigus vulgaris and bullous pemphigoid severe mucosal lesions and ulcerations are almost always encountered.
The pemphigus diseases are the result of autoantibodies bound to the skin and circulating in the serum. These antibodies target molecules crucial for the keratinocyte cell-to-cell adhesion. What initiates this auto-antibody formation is still unknown. Breed predilection or genetic factors have not been identified although some authors suggest that Quarter horse, Appaloosa and Thoroughbred breeds are overrepresented. Factors that have been suggested to be involved in the pathogenesis include contact with insects, drug provocation, UV-light and stress.

In practice, a tentative clinical diagnosis of pemphigus is often confirmed by a histological examination of a skin biopsy. Although a biopsy of an intact vesicle is preferred, this is often not possible as these vesicles are very fragile. Direct immunofluorescence or immunohistochemical staining may detect pemphigus antibodies but these techniques are not often available in diagnostic labs and have a relatively poor sensitivity and specificity. Microscopic examination of direct smears may reveal non-degenerate neutrophils and/or eosinophils and numerous acantholytic keratinocytes in cases of pemphigus foliaceus. However, incidental acantholytic keratinocytes may occur in any suppurative condition and are also sometimes seen in equine dermatophytosis. Routine hematologic examination (haematocrit, white blood cell count and differentiation, total protein concentration and differentiation, electrolytes and liver and kidney enzyme concentrations are invariably non-diagnostic, although occasionally mild alterations may be found.

**Pemphigus foliaceus**

Pemphigus foliaceus is the most common form of pemphigus in horses. The disease has been reported to occur in horses from 2 months to 25 years of age. The signs may start with annular thick crusts, annular erosions with or without epidermal collarettes, annular areas of alopecia, variable degrees of oozing, matted hair coat and scaling. Lesions may start at the head and limbs and spread over the body within a few months, but may also start on the trunk. In some cases the lesions are localised on the face or coronary bands for longer periods. Cases in our clinic are often reported to start with an urticaria-like skin problem, incidentally with leakage of serum and then progressing to severe crusting. The symptoms are often accompanied by depression, lethargy, some fever, filled/oedematous limbs and ventral oedema. In some horses oedematous limbs and ventral oedema are the first reported clinical signs. Pruritus and pain may or may not be present. The prognosis is poor.

Histopathology of pemphigus foliaceus typically reveals discrete pustules within the superficial layers of the epidermis containing mainly neutrophils, but eosinophils may be present, in association with acantholytic keratinocytes. The pustular changes may also affect infundibula of hair follicles. Because the pustules are transient in nature, the hallmarks of pemphigus foliaceus might not be present in skin biopsies. Removal of the pustule by thorough cleaning of the skin might also result in loss of the typical lesions in a biopsy.

**Pemphigus vulgaris**

Pemphigus vulgaris is extremely rare in horses. Vesicles, bullae and consequent painful ulcers occur in the mouth, on the head and neck and at the mucocutaneous junctions. A skin biopsy will reveal suprabasilar acantholysis, cleft and vesicle formation. The typical histopathological lesion of pemphigus vulgaris are suprabasilar clefts and formation of bullae with a tomb-stone like appearance of the basal cells at the basal part of the bulla. The prognosis is poor.
**Bullous pemphigoid**

Bullous pemphigoid is also extremely rare in horses and may clinically resemble pemphigus vulgaris although there is a discernible histological difference. Subepidermal vesicle formation is characteristic. Reported cases range from 5- to 14-years of age but the disease is very rare and horses of other ages may also develop the disease. In bullous pemphigoid the bullae are located just below the stratum basale of the epidermis. The bullae are filled with neutrophils, eosinophils fibrinous material, and mononuclear cells. There is no reported case of survival (despite intensive high dose glucocorticoid therapy).

**Differential diagnoses**

The differential diagnosis of pemphigus foliaceus based on clinic presentation includes dermatophytosis, dermatophilosis, bacterial dermatitis/folliculitis, sarcoidosis, multisystemic eosinophilic epitheliotropic disease (MEED), seborrhoea, cutaneous adverse drug reaction and epitheliotrophic lymphoma. Differential diagnoses of pemphigus vulgaris and bullous pemphigoid mentioned in literature include viral skin diseases e.g. equine pox virus, coital exanthema (EHV-3) and vesicular stomatitis, lupus erythematosus, drug eruptions, generalised granulomatous enteritis syndrome, chronic dermatophilosis, chronic chorioptic mange, oral irritations and toxic burns, and oral ulcers as a result of renal failure.

**Treatment of pemphigus foliaceus, pemphigus vulgaris and bullous pemphigoid**

Considering the poor to hopeless prognosis of pemphigus vulgaris and bullous pemphigoid, treatment is largely limited to pemphigus foliaceus cases. Younger horses may tend to have less severe disease than older horses. They may respond better to treatment and might remain in remission even after treatment is withdrawn. Some of the older cases do respond very well to treatment but a relapse may occur after weeks, months or even years.

The basis of all treatment is the use of long-term, high (immunosuppressive) dose, glucocorticoid administration (usually prednisolone per os or dexamethasone i.v. or i.m.). Oral omega fatty acids and vitamin E supplementation and sunlight restriction are also mentioned. Sometimes attempts are made to also address possible underlying causative factors such as fly bite allergies, drug administration, and diet.

Horses that do not respond to these medications may be treated with injectable gold salts such as aurothioglucose (1 mg/kg BW every 7 days for 3 weeks i.m.), azathioprine (3 mg/kg BW q 24h per os for 30 days and then half the dose for another 30 days), and/or oral pentoxifylline (10 mg/kg BW q12h).

The corticosteroid treatment is often started with dexamethasone i.m. (0.02 to even 0.1 mg/kg BW q24h) or with prednisolone per os (1.5-2.5 mg/kg BW q24h) and then tapering over several weeks to a maintenance dose (0.1 to 0.5 mg/kg/BW q24h or every other day per os). Bathing the horse in cool water might make it more comfortable. Unfortunately, many horses that initially respond to treatment suffer relapses, some immediately, others after weeks, months or even years. Furthermore, these horses may become progressively less responsive to treatment with each relapse. The prognosis is poor as horses may become refractory to treatment or may develop laminitis secondary to treatment. Additionally the treatment may become too costly for the owner.
Side-effects of the use of corticosteroids
Horses with pemphigus often require high doses of dexamethasone or prednisolone and owners are often worried about inducing catastrophic laminitis. Various epidemiological studies of risk factors and causes of laminitis have however shown that only a small minority could be attributed to the use of glucocorticoids. The exact mechanisms of cause and effect in the relationship between endogenous and exogenous corticosteroids and laminitis remain unclear. It is suggested that orally administered dexamethasone at a dose of 0.04-0.2 mg/kg can be used safely without risk of side-effects. However, in Utrecht we have poor experiences with oral dexamethasone.

At Utrecht University Equine Clinic prednisolone (1 mg/kg orally q24h always administered before 09:00h a.m.) has been used for over 35 years for different reasons with only very rare laminitic side-effects. In the few cases where laminitis occurred prednisolone had mostly been administered either twice daily or not in the early morning. The importance and advantage of the administration in the early morning is that the natural diurnal rhythm of cortisol/prednisolone is maintained.

Between 2008 and 2010 oral dexamethasone was used as an alternative choice of corticosteroid therapy. Twenty-one cases (10 Dutch Warmbloods, 11 different breeds; 8.4±4.5 years old) were treated for different indications with dexamethasone (orally 0.08-0.2 mg/kg q24h, before 09:00h) for varying periods of time (few days to several weeks). Eight of these cases (38%) became laminitic; four were on a dose of 40 mg/horse/day and four on a dose of 80-120 mg/horse/day. None of these cases had a prior history of laminitis and all were without an underlying problem that made the occurrence of laminitis more likely. As a result of this study oral dexamethasone is now no longer used in our clinic and instead either i.v. or im dexamethasone-dinatriumphosphate (0.04-0.14 mg/kg q24h before 09:00h a.m.) or prednisolone p.o. (0.5-1 mg/kg q24h before 09:00h a.m.) is prescribed.

![Mean cortisol concentration of 6 horses prior to any treatment](image)

Figure 1: Diurnal rhythm of endogenous cortisol concentration in 6 healthy adult horses.
Figure 2: Day-night rhythm of exogenous prednisolone concentration each day given at 07:30h a.m. in 6 healthy adult horses.

**Conclusion**
Cases with a diagnosis of pemphigus foliaceus, pemphigus vulgaris or bullous pemphigoid generally have a poor to hopeless prognosis. For that reason cases with a tentative clinical diagnosis of pemphigus require a thorough work-up and careful consideration of all differential diagnoses; many of the diseases in this differential list have a much better prognosis. Confirmation by histological examination of biopsies of sufficient quality is essential to establish the diagnosis categorically and to consequently give the owner honest and reliable advice.

Selected References

MY APPROACH TO NODULES IN THE HORSE  
(Excluding sarcoids)

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Equine cutaneous nodules encompass a large group of diseases that manifest as solid elevated lesions of the skin of greater than 1 cm in diameter, usually as a result of infiltration of inflammatory or neoplastic cells into the dermis or subcutis. Horses present with acute to chronic onset of single or multiple subcutaneous nodules, with or without draining tracts, and potentially accompanying systemic signs such as fever, cough, rhinitis, anorexia, and lethargy depending on the extent, location and metastasis of the disease. Nodules in horses can be broken down into 1) infectious, 2) inflammatory and 3) neoplastic categories. Sarcoids will be covered as a separate topic.

DIAGNOSTIC APPROACH
Although fine needle aspirates and skin cytology can be helpful to provide preliminary evidence suggestive of an etiology, nodular dermatoses require solitary to multiple incisional or excisional skin biopsies for histopathologic evaluation of the architecture and nature of the cellular infiltrate. In very thickened lesions or conditions where the pathology is suspected to involve the deeper tissues (e.g., chronic progressive lymphedema), a double punch technique is used to acquire samples with more diagnostic information; that is, an 8 mm punch biopsy of the upper epidermis is followed within the same opening by a 6 mm punch sample of the dermal and subcutaneous tissue.

To optimize the information obtained from the skin biopsies, it is recommended that one 8 mm aseptically acquired biopsy should also be submitted for tissue maceration and both bacterial (aerobic and anaerobic) and fungal cultures to rule-out the possibility of an infectious etiology; in particular, if draining lesions are noted.

Further diagnostic evaluation will depend on the disease process but may include special stains for infectious organisms or cutaneous structures, immunophenotype staining to identify cell types within a mass, and Polymerase Chain Reaction studies to identify DNA from potential infectious agents. Lastly, tumor staging would be indicated if a neoplastic condition is confirmed and should include routine lab work (CBC, clinical chemistry, urinalysis), radiographs (three-view thorax), lymph node aspiration for cytology review, and possibly advanced imaging (ultrasound, computed tomography).

Inflammatory Infectious Nodules
Fungal
Several fungal organisms can result in a nodular dermatosis ranging from intermediate to deep infections. These include intermediate infections such as saprophytes (Curvularia, Pseudoallescheria), phaeohyphomycoses (Bipolaris), zygomycoses (Basidiobolus, Conidiobolus), rhinosporidiosis, pythiosis, and sporotrichosis, to deeper and possibly systemic infections such as blastomycosis, histoplasmosis, coccidioidomycosis and cryptococcosis.
Geographic and regional microenvironment will narrow the list of the organisms for inclusion on a differential list. Although infections have been documented globally, the incidence of cutaneous fungal infections shows an increased propensity for more humid areas of the United States, South America and Australia. Two recent studies reported that fungal granulomas accounted for 10% and 9.4% of all non-neoplastic equine skin lesions in the US Pacific Northwest and the Southeastern Queensland region in Australia. Organisms are often introduced into the body via traumatic inoculation.

The disease typically affects young and older horses with lesions involving predominantly the distal limbs, head and neck region although any area on the body may be affected. Treatment may range from local extirpation of a nodule to systemic antifungal therapy in patients with more diffuse involvement. Schwarz et al in 2009 reported a 4-year-old horse with fungal granulomas involving the skin of the neck was successfully treated with a combination of oral fluconazole (Diflucan, Pfizer) for 10 days (14 mg/kg single loading dose followed by 5 mg/kg every 24h; based on Latimer et al pharmacokinetic data reported in 2001) with concurrent administration of oral potassium iodide at a dose of 30 mg/kg every 24 h for 30 days followed by diode laser resection of the fibrous tissue remnants, which were devoid of any fungal elements.

*Equine sporotrichosis* is a cutaneous and subcutaneous zoonotic mycosis caused by a dimorphic fungus *Sporothrix schenckii* that occurs as a yeast form in tissues and a telomorph form in soil, on bushes and trees. Infection in horses most often results from skin wounds infected with contaminated soil or plant material, causing cutaneous lesions that may progress to lymphadenitis (ulcerative lymphatic cording). The prevalence of sporotrichosis may have geographic differences. In 2009, Carothers et al reported in a retrospective study (1987-2007) from UC Davis in California that cats were more commonly diagnosed (14/23; 61%), while both dogs and horses each represented 17% (4/23) of the cases along with one donkey (1/23; 4%).

Diagnosis is made when the organism is detected on skin cytology (not as prevalent as in cats), histopathology, immunofluorescent antibody testing on affected tissues, and/or culture. The lesions are characterized by a pyogranulomatous inflammatory response with round, oval, or cigar-shaped, 3 to 5 mm wide and 5 to 9 mm long organisms that may be surrounded by a clear halo resembling a capsule.

Ethylene diamine dihydroiodide (organic iodide powder; EDDI) is the drug of choice for horses, especially considering cost of treatment. This product is in the form of a feed additive given orally at a dosage of 1-2 mg/kg of the active ingredient twice daily for the first week. The dosage is then reduced to 0.5 - 1.0 mg/kg once daily for the remainder of the treatment. Lesion regression on EDDI should be noted within one month and should be continued for 1 month beyond clinical resolution. Although the mechanism of action is unknown, EDDI is thought to boost the host's immune response and increase the elimination of the fungi through the skin or hair. Side effects seen are related to iodide toxicity (iodism) including excess scaling and alopecia, a serous ocular or nasal discharge, excess lacrimation and salivation, anorexia, depression, abortion and infertility, coughing, nervousness, or cardiovascular abnormalities (tachycardia). Treatment should be discontinued until adverse effects have resolved and then resumed at 50-75% of the dosage. Iodides should not be used in pregnant mares as they may result in severe congenital hypothyroidism with limb and other deformities in foals. In 2004,
Kohler et al demonstrated that both terbinafine and itraconazole are effective in vitro against *Sporothrix* organisms isolated from a horse. With the expiration of various antifungal patents, the feasibility of using oral azoles or allylamines may warrant further pharmacokinetic and clinical efficacy studies in horses.

Parasitic
Nodular dermatitis associated with parasitic infections may be attributable to tick bite granulomas, hypoderminiasis, halicephalobiasis, parafilariasis, hypoderminiasis and habronemiasis. **Habronemiasis** is a granulomatous disease of horses caused by the aberrant introduction of larvae by flies, their intermediate hosts, (the stable fly, *Stomoxys calcitrans* for *H. majus*; house fly, *Musca domestica* for *H. muscae* and *Draschia megastoma*) into wounds on distal extremities or mucosal regions such as the sheath or periocular region with an ensuing hypersensitivity and nodular granulomatous response. Donkeys and mules develop very large lesions. Differential diagnoses for granulomatos nodular reactions involving the limbus of the eyes should include equine sarcoid, squamous cell carcinoma, phycomycosis or onchocerciasis. Generally, however, *Onchocerca cervicalis* transmitted by *Culicoides sp.* tends to cause a more diffuse dermatitis along the neck and ventral midline versus a nodular dermatosis. Be aware that skin infections and cutaneous neoplasms can also become secondarily infected with *Habronema* larvae, hence histopathology is key to ruling-out concurrent conditions. The prevalence of habronemiasis varies considerably throughout the globe and may be negatively influenced by the regular use of deworming agents. However, with the recent shift to limit use of dewormers to only high shedding individuals based on fecal evaluation, a resurgence of habronemiasis and onchocerciasis may be imminent.

Diagnosis is based on history, clinical signs, presence of sulfur granules, and cytologic or histopathologic identification of larva(e). Histopathologic findings typically include granulation tissue with focal to diffuse infiltration of eosinophils and areas with coagulation necrosis surrounding a centrally located larva. The eggs are not usually identified in feces because they have thin walls, which readily collapse.

Ivermectin (0.2-0.3 mg/kg) has been shown to be effective and is considered the treatment of choice to address the larvae. Due to the hypersensitivity reaction that accompanies the infection, anti-inflammatory systemic oral (prednisolone 1 mg/kg q 24 h for 14 days and then tapered), intralesional (3-5 mg of triamcinolone/lesion; max dose of 20 mg to avoid steroid-induced laminitis) and/or topical corticosteroids are prescribed. In severe cases, surgical removal or debulking of the lesion should be considered. Strict attention to fly control and wound management will minimize recurrence of the lesions.

Bacterial
Bacterial infections typically result in papular eruptions and folliculitis with resultant hairloss. Several bacterial conditions are more likely to cause nodular dermatoses. These include actinomycosis, nocardiosis, staphylococcosis, tuberculosis, glanders and *Corynebacterium pseudotuberculosis* infections. *C. pseudotuberculosis* infections usually present as solitary or multiple abscesses or nodules with many draining tracts that progress to diffuse cellulitis. Insect vectors include stable, horn and houseflies. The distribution of the nodules and abscesses include the pectoral region ("Pigeon Fever"), face, neck, axilla, groin, and limbs. The lesions often
rupture in 1-4 weeks releasing creamy purulent exudates, which act as a major source of contamination.

Treatment of solitary lesions involves hot packing, bringing the lesions to a head and cleaning with chlorhexidine once ruptured. If multiple lesions are noted and in regions of the body that are difficult to drain, institution of systemic antibiotics may be warranted. Trimethoprim sulfa (30 mg/kg, q 12 h, PO) or ceftiofur (2.5-5 mg/kg IV q 12 h) with rifampin (3-5 mg/kg, PO) may require a minimum of one month and sometimes up to 6 months to resolve the infection. Consider using concurrent probiotics to minimize the risk of colitis associated with protracted use of antibiotics.

Inflammatory Sterile Nodules
Once a nodular lesion has been determined to be non-infectious in nature, several considerations include axillary nodular necrosis, unilateral papular dermatosis, proud flesh, hematoma, sterile nodular panniculitis, foreign body granuloma nodular auricular chondropathy, pseudolymphoma, calcinosis circumscripta, myospherulosis, and the most frequently diagnosed eosinophilic granuloma. Lesion distribution and histopathology should provide a definitive diagnosis.

Eosinophilic Granuloma (Nodular Necrobiosis)
Eosinophilic granulomas are the most common non-neoplastic nodular disease in horses, characterized by intense eosinophilic infiltrates. The collagen degeneration that accompanies this condition is most likely due to release of toxic eosinophilic contents such as major basic protein. Similar to cats, eosinophilic granulomas tend to be a reaction pattern attributable to an allergic etiology. A large subset of affected horses represents hypersensitivity reactions to insect bites. Evidence to support this hypothesis includes the fact that:

- Many affected horses have been diagnosed with *Culicoides* hypersensitivity
- Nodules recur each year with the onset of pruritus and the insect season and tend to resolve in the winter or with insect control
- Lesions occur at body sites on which insect feeding has been documented

Other groups of affected horses were intradermal allergy test positive for inhalants, but not insects, whereby allergen specific immunotherapy (ASIT) resulted in resolution of clinical signs, suggesting atopic dermatitis as a potential underlying etiology. Food allergy has also been proposed as dietary trials have resulted in resolution of clinical signs and confirmed by dietary challenges resulting in relapses. Injection site granulomas were reported in response to the silicone coating on hypodermic needles; future reactions were avoided by using uncoated stainless

As mentioned previously, nodules usually appear in the warmer months of the year, although geographic variations exist, and males have been found more frequently affected. One or multiple lesions, which vary in size from 1-10 cm, typically are round and firm, with no hyperpigmentation, alopecia or ulceration noted. Atypical lesions may ulcerate and drain, while some may be cystic or plaque-like, with a central caseous or calcified core. The neck, withers, saddle, and girth are the most affected areas. Multiple lesions (sometimes hundreds) on one side of the body only have been rarely reported.
History, palpation and clinical appearance are very suggestive of this condition. Confirming the diagnosis requires dermatopathologic evidence of a granulomatous reaction and the appearance of flame figures around collagen bundles consisting of eosinophils and eosinophilic granules or “mush”. Calcification can be observed in older lesions.

Glucocorticoids are the principal means of treating these lesions. If a single lesion is noted, intralesional or sublesional injection of 5 mg triamcinolone acetonide q 2 weeks for 3 treatments provides a non-surgical alternative. If an incomplete resolution is noted with this protocol, or concern regarding laminitis and other adverse effects associated with the use of glucocorticoid exists, surgical extirpation or CO₂ laser ablation should be considered. When multiple lesions are present, prednisolone 1-2 mg/kg/day 7-10 days, then tapering completely off medication within 3-4 weeks is likely with this condition especially if the underlying etiology is addressed by ectoparasite control, dietary trial and ASIT.

Neoplastic Nodules
Squamous Cell Carcinoma
Squamous cell carcinoma (SCC) is the second most common tumor diagnosed in horses (next to sarcoids), accounting for 20% of equine neoplasms. It commonly affects the non-pigmented mucocutaneous junctions including the ocular conjunctiva and the external genitalia but can also involve other areas of the body including the perioral and perinasal region, the aural canal, and perianal tissue. Local metastasis to the lymph nodes has been noted in 18.6% of cases. The regional lymph nodes should also be assessed for metastasis by palpation, inguinal and rectal examination, and (ultrasound-guided if available) fine needle aspirate. SCC may also spread to the lungs. Breeds more commonly affected include draft breeds, Appaloosa, American paint and pinto horses. The presence of smegma, persistent phimosis, or repeated trauma may predispose stallions and geldings to developing lesions of the penis or sheath. Regular examination and washing of the prepuce and penis will aid in early detection of SCC and may even assist in preventing its development. SCC affecting female genitalia may involve both the vaginal vestibule and mammary glands. Early clinical signs of SCC include thickening, hypopigmentation, exfoliation and ulceration of the skin. Differential diagnoses include sarcoids, melanoma, exuberant granulation tissue and pythiosis.

Early recognition is key to a successful treatment outcome for SCC. A combination of surgical debulking and chemotherapy are recommended such as traditional surgical methods, CO₂ laser ablation or cryosurgery, along with post-operative chemotherapy (5-fluorouracil ocular drops or creams, or intralesional 5-fluorouracil with epinephrine [10:1 respectively] at doses of 1–3 ml per tumor site). Maximum systemic dosage is 750 mg for the average horse. Similarly, aqueous cisplatin can be injected or beads (1.6mg/bead) placed intralesionally to a maximum systemic dosage of 100 mg. Radiation therapy using strontium-90 (ocular) or cobalt 60 (paranasal) radiotherapy has provided good to excellent long-term resolution. Recurrence of the tumor is common, especially if restriction to predisposing factors, such as solar radiation, smegma, and trauma, cannot be limited. Thoracic metastasis clinically presents with progressive weight loss, anorexia, and intermittent fever and should be confirmed by chest radiographs. If present in the lungs, the owner should be advised of the poor prognosis before continuing further treatment.
Mastocytosis
Mastocytosis (mast cell tumors) occurs in horses 1 to 18 years of age (mean = 9 yr), with no confirmed sex or breed predilection. Multiple mast cell tumors resembling urticaria pigmentosa of humans may occur in newborn foals; these spontaneously appear and regress. Equine mastocytosis is usually solitary and occurs most commonly on the head and trunk, but may involve the distal limbs. Lesions are 0.5 - 20.0 cm in diameter, well to poorly circumscribed, firm to fluctuant, dermal or subcutaneous, and may or may not be alopecic, ulcerated, and hyperpigmented.

Histology may vary from sheets of mast cells with few to numerous eosinophils with or without collagen degeneration. Parasitic conditions such as onchocerciasis should be eliminated as a differential by treatment with ivermectin. Clinically, most mast cell tumors in horses do not recur after being excised and rarely do they metastasize bringing into question the neoplastic versus dysplastic nature of this condition. Surgical removal is the treatment of choice and recurrence is rare. Intralesional corticosteroids might be beneficial for areas where surgery may be difficult or disfiguring.

Melanoma
Melanocytic skin tumors of horses traditionally have been described in aging grey horses, in typical locations of the ventral tail, perineum, external genitalia, lip, udder, periorbital and parotid gland regions. Both graying and melanoma formation have been linked to a duplication in the Syntaxin 17 (STX17) gene, with the STX17 genotype affecting melanoma grade and severity in Lipizzaner horses. An agouti signaling protein (ASIP) mutation, responsible for bay/black coat color, modifies melanoma risk in gray horses with increased melanoma severity potentially due to an increase in melanocortin 1 receptor (MC1R) pathway signaling. Most tumors are benign accumulations of melanophages filled with melanin. It has been suggested that this is really a storage disease rather than a true neoplastic condition. They have been the subjects of several classification schemes in attempting to correlate histopathologic appearance with clinical behavior (i.e., is it benign or malignant). Three basic types of melanocytic skin tumors have been noted:

_Melanocytic nevi (melanocytoma)_ occur in the superficial dermis or at the epidermal-dermal junction, frequently have epithelial involvement, with nests of relatively large, mildly to moderately pleomorphic cells showing variable cytoplasmic pigmentation and occasional mitoses. More than 70% of these occur in horses less than six years of age, and may occur in horses of any color (not just grey). Most of these tumors occurred in atypical locations. Of 28 melanocytic nevi, only one became invasive, while the rest exhibited benign behavior.

_Dermal melanomas_ are found in the deep dermis, and are composed of small homogeneous, indistinct tumor cells, either round or dendritic, with no mitoses. (If there are multiple, confluent dermal melanomas, this is referred to as dermal melanomatosis). 80% of these tumors are in horses older than six years of age or between 5-15 years and are much more common in grey horses. Most of these tumors occurred in typical locations. Of 14 cases available for follow-up in one study, 8 had malignant behavior as demonstrated by metastases.
Fleury et al in 2000, reported the clinical and pathological characteristics of cutaneous melanomas occurring in 83 Camargue-type gray-skinned horses showed that the tumors occurred most frequently underneath the tail (93.9%) and at high rates in the perianal region (43.0%), the lips (33.0%), and the eyelids (24.0%), but rarely in the vulva (3.8%). Microscopic examination indicated that these tumors were composed mostly of melanocytes and numerous melanophages and that these cells manifested a remarkable cellular atypia. Early stages of the tumors occurred in close association with apocrine sweat glands, but not at the dermal-epidermal junction.

Seltenhammer et al in 2003 conducted a clinical study on 296 grey horses of the Lipizzaner breed. Of the 296 horses, dermal melanomas were present in 148 horses (50%), 68 of which were more than age 15 years; 51 of these were melanoma-bearing. In 75.6% of cases, melanotic tumours were detected underneath the tail. None of the affected individuals suffered any severe clinical effect or was handicapped in performance. The authors concluded that in contrast to melanomas in solid-colored horses characterized by early metastases, melanomas in grey horses showed less malignancy. Affected individuals often had encapsulated nodules or structures similar to human blue nevi. This finding partially reflects confusion in terminology between true malignant melanomas and dermal melanomas.

Anaplastic malignant melanomas are composed of sheets of extremely pleomorphic epithelioid cells with poor pigmentation and many mitoses. These are usually seen in horses older than 20 years of age and in horses of any color.

Melanomas are malignant neoplasm with the capacity for local invasion and metastasis. Excision of dermal melanomatosis from the perineal, perianal, perirectal, or ventral tail regions will often provide a favorable outcome. Cryosurgery can be used in conjunction with surgical excision. However, tumor regrowth is possible. Post-surgical chemotherapy may help to minimize recurrence. Intrallesional injection or implantation of cisplatin has been very effective in the treatment of tumors less than 3 cm in diameter.

Histamine stimulates T suppressor cell inhibition of both cell-mediated and humoral immunity. Cimetidine appears to block histamine H2 receptors on these cells, thereby enhancing immune function and targeting of these tumors. Unfortunately, cimetidine (2.5-5 mg/kg po q8-12h for a minimum of 16 weeks and continued for 2–3 weeks following resolution of tumor growth) has been shown to decrease the number and size of melanomas inconsistently, most likely due to its poor oral bioavailability (14%). To achieve concentrations associated with efficacy in humans, an unaffordable dose of 48 mg/kg is required. Adding piroxicam (80 mg PO q24h for 500kg horse) to cimetidine therapy may be beneficial but there are currently no controlled studies of this therapy.

Other non-conventional treatments have been proposed. Transdifferentiation, or the conversion of one differentiated cell type into another, was used successfully to convert malignant ocular melanoma to benign cells in a 22-year-old, grey hair Welsh pony. Adenosine was injected subconjunctivally as a transdifferentiation agent and an adenosine agonist was then given as eye drops daily for two weeks. Then, retinol was injected subconjunctivally. Two weeks after the retinol injection, dramatic shrinkage of the tumor was observed that circumvented the need for surgical enucleation.
In 2011, Müller et al reported successful outcomes when using cytokine encoding plasmid DNA (IL 18 and IL 12) injected intratumorally into horses with metastatic melanomas as compared to a placebo controlled group (empty plasmid DNA).

A novel therapeutic DNA plasmid vaccine encoding human tyrosinase (pING-HuTyr; Oncept®) has been developed for use in dogs. This xenogeneic therapy (using DNA from one species to elicit a response against another species) significantly improved survival compared to historical controls in a prospective, 5-site, USDA-regulated, 110-dog study. This is the first US government-licensed therapeutic vaccine for the treatment of cancer in either animals or humans. Importantly, the vaccine is safe and with side effects rarely noted. The vaccine is administered via a new "needleless" transdermal device. The vaccine protocol requires four (4) treatments on an every-other-week basis and booster vaccines every 6 months. Antigen-specific immune responses have been seen in all species investigated to date including mice, rats, dogs, and humans. Phillips et al in 2012 noted that tyrosinase is produced in equine neoplastic melanomas at high quantities, similar to humans and dogs, and that the equine tyrosinase sequence has a 90% homology to the human sequence. Based on this information, the transdermal injection of the xenogeneic tyrosinase DNA vaccination would be suitable for use in horses. Phillips and Lembcke in 2013 reported evidence of tumor shrinkage with vaccine use in over 50 horses. Positive humoral responses were seen in all treated horses. Cellular reactivity was noted with tumor infiltrating lymphocyte populations identified a statistically significant increase in CD8+ lymphocytes along with a decrease in CD4+/Foxp3+ regulatory T cells following vaccination. Increasing plasmid dose did not appear to be associated with an increase in either clinical activity or immunologic reactivity. This xenogenic vaccine appears to be safe and well tolerated in tumor-bearing horses and appears to result in both clinical activity and a measureable immune response in treated patients. Larger clinical trials are currently underway in horses along with use of other plasmid-based DNA vaccines (e.g., Streptococcus pyogenes emm55 gene).

Cutaneous Schwannomas
Benign peripheral nerve sheath tumors are a diverse group of neoplasms that includes schwannomas, neurofibromas, perineuriomas and ganglioneuromas. In 2011, Schöniger et al described a review of 22 cases of equine schwannomas that were submitted for histopathologic evaluation between 1979 and 2008. Horses were 8 to 25 years old with no evidence of sex predilection. The horses had solitary cutaneous masses with the majority occurring on the head and neck with involvement of the trunk, extremities and tail rarely. The tumors were well demarcated, expansile, multinodular to solitary nodular lesions located in the dermis. Densely packed spindle-shaped neoplastic cells were arranged in short fascicles with nuclear palisading (Antoni A). Hypocellular regions of the tumor revealed neoplastic cells separated by abundant myxomatous stroma (Antoni B). Tumors commonly had cellular vacuolation, along with myxomatization of stroma and vessel walls. Neoplastic cells were immunopositive for S100 protein. Surgical excision of the tumor is the treatment of choice offering a good prognosis with little chance of recurrence.

Cutaneous Lymphoma
Cutaneous lymphomas are rare in horses but are the most common malignant equine neoplasms. The age of onset ranges from 2 months to 31 years (mean, 10.7 years). Quarterhorses are the
most common breed, followed by thoroughbred and Standardbred horses. Equine cutaneous lymphoma is primarily divided into two categories: a) cutaneous T-cell lymphoma (CTCL), an epitheliotropic generalized scaling dermatosis seen most often in Thoroughbreds compared with other breeds and, b) T-cell-rich, large B-cell cutaneous lymphoma (TCRLBCL) characterized by non-epitheliotropic solitary to multiple nodules affecting the entire body with a predilection for the eyelids and more often noted in quarter horses. Less common lymphoma subtypes include diffuse large B-cell lymphoma and anaplastic large T-cell lymphoma.

Unlike epitheliotropic lymphoma (CTCL) where the prognosis is grave, survival rate post-diagnosis of non-epitheliotropic lymphoma (TCRLBCL) may vary between months to years. It is suggested that these tumors are more frequently of the lymphohistiocytic type, potentially attributable to an antigenic stimulus or an ovarian granulosa cell tumor. Seasonal recurrence and regression, along with amelioration of the nodules with removal of the tumor or supplementation with progestins, raises speculation as to whether the non-epitheliotropic form of lymphoma should be reclassified as pseudolymphoma. Another reported cause TCRLBCL histopathologic findings includes Borrelia infection transmitted by tick bites that responded positively to treatment with doxycycline. As well, PCR analysis revealed higher positive findings for EHV-5 in affected individuals in comparison to normal, which suggest that EHV-5 may be an etiologic agent associated with the development of some types of equine lymphoma.

In general, prognosis declines when metastasis to regional lymph nodes is noted along with rapid weight loss, lethargy, ventral edema and pyrexia. Metastasis rarely involves internal body organs. Diagnosis is based on immunohistologic confirmation that the nodules are composed of densely packed lymphoblastic cells expressing CD79a, and numerous small, round, CD3-positive T lymphocytes. Abnormal circulating lymphocytes are seen in 25–50% of cases. Decreased IgM level is often seen in horses with lymphoma; however, the sensitivity is poor (~28%) while specificity is fairly good (~88%). Other than progestins and surgical removal of ovarian tumors, therapy may involve identifying and eliminating the antigenic stimulus, considering antiviral therapy, or prednisolone (1-2mg/kg/day with taper) and/or lomustine chemotherapy (60-90 mg/m² q30d).

Take Home Messages:
1) There are both infectious and non-infectious causes of cutaneous nodules. As well, many equine tumours look alike or may mimic other conditions. Definitive diagnosis is key to establishing a prognosis and providing appropriate therapy.
2) Although newer remedies are emerging, the clinician must also be aware of older remedies that have continued efficacy in the treatment of equine cutaneous nodules.
3) Aseptically prepared surgical skin biopsies taken for culture (aerobic, anaerobic and fungal) followed by multiple skin biopsies for dermatohistopathologic evaluation will help rule-in or rule-out infectious etiologies, as well as, provide better diagnostic and prognostic capabilities, especially in the hands of a pathologist with a focus on equine skin disease.

Selected References


EQUINE SARCOIDS: UPDATE ON THE MOST COMMON EQUINE TUMOUR

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Introduction

The sarcoi d is a neoplasm largely restricted to the horse that results from proliferation of fibroblasts; dermal cells that produce connective tissue (e.g. collagen, elastin, extracellular matrix) important to skin structure and resilience. The term sarcoi d was first used in 1936 by Cecil Jackson from South Africa, defining it as “a fibroblastic tumor-like condition of equine skin with a variable epithelial component and high propensity for recurrence.” It is the most common equine skin neoplasm ahead of squamous cell carcinoma, melanoma, and papilloma, with prevalence from 1-12% reported. Sarcoi ds occur in other equids (donkeys, mules, zebras), and a histologically similar disease occurs rarely in cats. Although sarcoi ds do not metastasize, they remain a therapeutic challenge due to variable clinical presentation, locally aggressive behavior, and high recurrence rates following surgical excision. Euthanasia is not uncommon due to the prolonged nature and cost of treatment, and risks of recurrence.

What causes sarcoi ds?

Aetiopathogenesis of the sarcoi d is incompletely characterised, and appears complex and multifactorial. Environmental and genetic factors are implicated to influence initial development and progression of disease. Despite a likely infectious origin, variation in individual susceptibility is apparent. Bovine papillomaviruses (BPV) are strongly implicated as causal agents, with BPV DNA detected within nuclei of tumor fibroblasts, viral proteins consistently detected by PCR within sarcoi ds (often in large amounts), and similar lesions reproduced experimentally by virus inoculation onto scarified skin. However, actual viral elements (virions) are as yet to be detected on electron microscopy studies. Induction of experimental lesions in horses has also proven difficult and as yet is not accurately reflective of spontaneous disease, with induced lesions having delayed regression linked to development of BPV antibodies. BPV DNA has also been detected in the skin (more prevalent in inflamed skin) and blood of unaffected horses not in contact with cattle or sarcoi d-bearing horses, and in adjacent normal skin of horses with sarcoi ds, but not in other skin tumors or equine papillomas. Fibroblastic tumors distinct to sarcoi ds have been experimentally induced by inoculation of BPV in mice and hamsters. Thus complete confirmation of disease aetiolog y has not been possible to date. There remains question over the ability of BPV to replicate in the horse, and whether the virus has truly adapted to a new host.

Viral Infection Papillomaviruses are widespread and generally species-specific, with a variety of subtypes infecting many animal species (mammals, birds, reptiles; over 100 subtypes in man alone). Papillomaviruses have known oncogenic potential, and usually induce benign lesions of cutaneous and mucosal epithelia, especially of thin or traumatised regions. Most skin infections are restricted to the epidermis, and most lesions are benign and regress spontaneously following activation of a host immune response, but occasional forms evade host immunity and persist, and
some develop into more aggressive forms of neoplasia (e.g. SCC). Bovine papilloma viruses (BPV) have at least 10 different subtypes, each associated with different clinical forms of papilloma in cattle. BPV-1 and BVP-2 are now considered the most important aetiological factors in the development of equine sarcoids. This represents an atypical cross-species infection of papillomavirus. Cross-species infection is also reported with BPV in bison and buffalo. Histologically similar sarcoids in cats are also linked to papillomaviruses distinct from, but most similar to BPV.

BPV-1 and BPV-2 belong to a distinct group of papillomaviruses that differ from the majority of papillomaviruses in their ability to infect dermal as well as epidermal cells, and thus give rise to distinctive fibropapillomatous lesions. In cattle BPV-1 is associated with fibropapillomas of the genitalia, and BPV-2 is associated with cutaneous verrucous papillomas, fibropapillomas of gastrointestinal tract, and transition to aggressive carcinoma of the urinary bladder. Horse-specific mutations of BPV-1 genes have been detected in sarcoids, representing an adaption of this virus to the horse. BVP infection in horses was initially considered abortive, with virus residing exclusively in fibroblasts and not multiplying in the epidermis as for most papillomaviral infections. However, accumulating evidence for transmission between horses suggested productive infections, and DNA from BVP-1 has now been detected within sarcoid epidermis, albeit at lower levels than occurs with infection in the normal bovine host. There may be variation in BVP subtypes involved in sarcoids in different regions; BVP-2 has been more frequently associated with sarcoids in Canada, and BVP-13 DNA recently detected in sarcoids in Brazil.

In contrast to infection with BPV, equine papillomavirus (EPV) infection in horses is confined to the epidermis and associated with formation of small, circumscribed, verrucous papillomas. Viral replication within the epidermis and high viral load are readily detectable. Seven subtypes of EPV have been identified, with more than one type potential within lesions. Clinical forms recognized included genital papillomas, aural plaques, and the common muzzle/lip verrucous form (associated with EcPV-1; one of the first forms shown to be transmissible). Equine sarcoids appear associated with papillomaviruses more adapted to the bovine host, with a complex interaction of host and unusual viral adaptation involved in disease production.

Genetics Evidence to support a genetic predisposition to sarcoids is accumulating; however, heritability and the mode of inheritance remain unknown. There is increased prevalence in certain families of horses, and varying prevalence amongst different breeds (more common in quarter horses and less common in standardbreds compared to thoroughbreds). An increased risk of disease in some breeds is linked with certain genetic elements (e.g. Equine Leukocyte Antigen [ELA] W13). Recent genomic testing has suggested a polygenic inheritance pattern, with candidate regions identified on equine chromosomes that include genes regulating virus replication and host immune response. A moderate heritability of 21% was proposed in one study of over 300 affected Franches-Montagnes horses in Switzerland, and restricted breeding raised as a potential method for reducing disease incidence.
How are sarcoids produced?

Skin trauma Papillomaviruses cannot actively penetrate intact skin, and infection requires some form of skin trauma. Sarcoids are frequently reported to occur at sites within 3-6 months of traumatic or surgical skin damage.

Viral transmission Papillomaviruses are resistant to drying or freezing, and can persist in the environment for at least three weeks. High temperatures, detergents, and formalin can reduce virus survival. Papillomavirus transmission occurs via direct contact, and for some subtypes with ample viral load, transmission is confirmed via fomites (e.g. HPV-1, HPV-2 causing classical humans verrucous ‘warts,’ BPV-1 in cattle causing classical bovine verrucous ‘warts,’ and EcPV-1 in horses causing classical equine verrucous ‘warts’). For papilloma lesions associated with low-level virus replication (e.g. HPV-16, HPV-18 causing human genital papillomas, and BPV-1 in horses causing equine sarcoids) a confirmed role for indirect transmission via fomites is lacking despite supportive evidence. In relation to BPV infection in horses, epizootic outbreaks of sarcoids and apparent transmission from affected to non-infected in-contact horses/zebras are reported, and transmission has long been suggested both from BVP affected cattle to horses, and between horses. Direct horse-to-horse transmission is supported by the association of a majority of sarcoids from horses in Europe and UK with equine-specific BPV mutations. Insect transmission is proposed, and BPV-1 identical to that detected in sarcoids from horses has been isolated from biting and non-biting flies in the same regions. However, sarcoids occur worldwide, with no apparent seasonal or geographic predispositions identified so far, suggesting insect transmission as only one possible route of viral transfer.

Viral transformation

As discussed, in contrast to classical papillomavirus infections, the fibroblast is the main cell altered in sarcoids. BPV has been shown to alter the expression of several genes in transformed fibroblasts. A range of studies have also documented potential roles for enzymes (e.g. matrix metalloproteinases) in facilitating dermal invasion of neoplastic cells, chemokines (e.g. CXCL5) for facilitating sarcoid growth, and increased regulatory proteins (IL-10, FOXP3) and transforming proteins (E5) for inhibiting cell immune responses to invading virus. Further studies evaluating conditions that allow tumor growth and persistence are underway.

Clinical presentations

Signalment One fairly unique characteristic of sarcoids is a predilection for young horses, with most disease occurring in horses from 3-6 years of age. Disease is suggested to be very rare for horses less than 1 year of age, or greater than 7 years. Many breeds can be affected, and although early studies suggested no clear breed predispositions, quarter horses appear to have greater risk, and standardbreds lower risk of disease. Thoroughbreds, Appaloosas, and Arabians may also be at increased risk, and higher incidence in some groups of related horses. Most studies suggest that there is no apparent gender or coat-colour predisposition.

Clinical Forms Another unusual feature of the sarcoid is its variety of clinical forms. A number of types have been suggested, although mixed forms are common, and progression from subtler to more aggressive types recognized.
The **occult sarcoid** most typically presents as focal, roughly circular, non-raised areas of alopecia that often contain small foci of scaling, mild lichenification, or small papules. They may also first present as focal areas of subtle hair colour or quality change. They may remain static for years, or slowly enlarge; many are reported to progress to verrucous forms. Less frequently they may develop rapidly into fibroblastic lesions, which has been purported to occur with trauma. The **verrucous sarcoid** has an irregular raised papillomatous and scaly appearance, and may be sessile (flat) or pedunculated. Lesions may be focal or coalescing over extensive regions, which are often surrounded by a zone of mild lichenification with altered hair quality. They are often slow growing, but similarly to occult lesions, purported to rapidly progress at times following trauma.

The **nodular sarcoid** is a more classical well-defined nodule of variable size (0.5-20cm or larger) that may be solitary, in small clusters, or occasionally occur in myriad clusters with thousands of lesions. They may occur as solely subcutaneous forms, with freely moveable overlying skin, or have dermal involvement. Overlying skin remains intact unless nodules increase dramatically in size or progressive rapidly to fibroblastic forms. Nodular sarcoids are also purported to progress to fibroblastic forms following trauma.

The **fibroblastic sarcoid** is a more locally invasive irregularly nodular lesion with prominent ulceration and exudation, which can also present as pedunculated or sessile forms. The depth of involvement of sessile forms can be far greater than suggested clinically. Lesions frequently resemble exuberant granulation tissue, and may develop at sites of previous trauma and slow-healing wounds. Fly worry, secondary bacterial infections, and myiasis are not uncommon.

The **malevolent or malignant sarcoid** is a rare aggressive invasive irregularly nodular lesion that may infiltrate lymphatics, and potentially involve local lymph nodes.

**Distribution**  
Sarcoids can occur in any body region but are most common on the head (pinnae, lip margins, periocular), neck, lower limbs, and ventral body (inguinal, perineal regions). Occult lesions may be more common in less-haired body regions. Verrucous forms may occur less commonly on the limbs except for coronary band areas. Sarcoids that develop at wound sites on the trunk are suggested to more typically develop into verrucous sarcoids, while those that develop at wound sites on limbs may be more typically fibroblastic.

**Differentials: what diseases may look like sarcoids?**  
Due to the wide variety of clinical presentations, there are a variety of skin diseases that are important differentials for sarcoids. Despite the frequent clinical assertion that diagnosis can be based on characteristic clinical appearance, recommendations are anecdotal, with limited scientific evaluation of clinical presentations of sarcoids. For occult presentations, infectious folliculitis (bacterial or dermatophyte), dermatophilosis, pemphigus foliaceus, and alopecia areata are potential causes of similar alopecic lesions. Papilloma, developmental hamartomas, or squamous cell carcinoma may mimic verrucous forms. For nodular and fibroblastic lesions there is a wide range of differentials, including infections (bacterial, fungal, habronemiasis, pythiosis), sterile inflammation (exuberant granulation tissue, foreign body reactions, eosinophilic granuloma), cyst formation (dermoid or follicular cysts), and other neoplasms (fibroma/fibrosarcoma, melanoma, neurofibroma)
Diagnosis: is biopsy a risk or important?
A definitive diagnosis of sarcoid requires histopathology. Historically there has been frequent reluctance to biopsy many suspected sarcoids due to accepted risks of biopsy-induced exacerbation of lesions. However, risks remain largely anecdotal, and the frequency of exacerbation of biopsied lesions is unknown. Biopsy of potential occult or verrucous lesions is indicated, especially if owners have concerns over lesion progression and agree to treatment if sarcoid is confirmed. Biopsy should be considered vital to distinguish between differentials for solely nodular or fibroblastic presentations, and is similarly important for non-healing wounds. Care should be taken to collect multiple sections from different depths of large lesions, as multiple aetiological agents may be present concurrently (e.g. exuberant granulation tissue, sarcoid, secondary bacterial infection). Mixed presentations may be more readily suggestive of sarcoids, although firm diagnosis still relies on histopathology.

Histopathology classically reveals a biphasic tumor with epidermal hyperplasia and dermal proliferation of transformed fibroblasts, although epidermal changes may be absent. Epidermal changes include hyperkeratosis and epidermal acanthosis with elongated truncated rete ridge projections into the dermis. Classical papillomavirus changes in the epidermis, including swollen pale keratinocytes and inclusion bodies are lacking. Dermal changes are dominated by a proliferation of immature fibroblasts with mitotic figures in a whorled fibrocellular mass, and alignment of neoplastic fibroblasts perpendicular to the epidermis in a ‘picket fence’ pattern is frequently cited. Differentiation from fibroma or fibrosarcoma may be challenging in some cases. PCR testing for BPV may be helpful as supportive evidence.

Surface skin cytology and fungal culture of alopecia lesions, and fine needle aspiration of nodular lesions may suggest or confirm alternate diagnoses without the need for biopsy, and are ideal to precede biopsy collection.

What are the practical treatment options?
Although a variety of treatments can be successful in eliminating or controlling sarcoids, there are no uniformly effective treatment options. There are also few controlled studies documenting comparative treatment efficacies, and as spontaneous regression of one or more sarcoids is reported in 30% of horses in some studies, optimal treatment regimes are currently unknown. Prevention of equine sarcoid may be facilitated by future development of vaccines against bovine papillomavirus.

Surgical excision is ideal whenever possible, however high recurrence rates (50-70%) are reported within 6 months for all except small, defined lesions. Determination of sufficient margins can be difficult (minimum 1.5 to 2cm recommended), and lesions on extremities in particular may not be readily excised. PCR testing for remaining BVP DNA has been investigated to guide sufficiency of excision, and although viral load may correlate with disease severity, residual viral DNA in margins does not appear predictive of recurrence potential. Cryotherapy or Laser therapy have been effective for smaller lesions not readily surgically excised, but scarring is frequent, especially over bony regions, and overall success appears low. Chemotherapy Topical 5-fluorouracil has been used effectively for treatment of papillomavirus lesions in humans (genital, plantar warts), either alone or after local surgery, although may produce problematic ulceration. Intrallesional injections of 5-fluorouracil were utilized effectively
for occult and verrucous sarcoids in one small study of 13 horses. Injections were repeated every 2 weeks for up to seven treatments with horses under sedation. The technique required “a large amount of pressure” when injecting. Resolution was reported in nine horses (61.5%; 3-year follow-up). Smaller and previously untreated lesions were more likely to resolve. Effective intralesional treatment with 5-fluorouracil is also reported in zebra. Response to topical 5-fluorouracil treatment is not reported. Intralesional cisplatin has been successfully used, but has significant exposure risks for administering veterinarians.

**Immunostimulation** Topical imiquimod 5% gel (Aldara, 3M) is indicated for human genital papillomas and has been used in small sarcoids in horses, with 9 of 15 tumors (60%) resolving completely within 8-32 weeks when applied three times weekly. Mycobacterial products (commercial whole-attenuated BCG) have been used intralesionally with apparent success in periocular sarcoids. However, post-injection inflammatory reactions were common, and fatal anaphylaxis reported. Autologous vaccines, produced utilizing excised sarcoid tissue frozen in liquid nitrogen, and subsequently implanted subcutaneously in the neck, have been successful (~65% resolution) in two small studies of 15 horses. Complications reported include swelling and occasional abscessation at sites of implantation in ~50% of horses.

**Antiviral treatments** Aciclovir 5% cream was applied daily for 2-6 months in 22 horses with 47 sarcoids, with complete regression in 32 sarcoids (68%). This modality may follow surgical debulking. Cidofovir 1% gel applied once daily for one month was effective in 2 of 3 horses with suspect sarcoids after surgical debulking. The non-responsive lesion was ultimately confirmed as SCC on biopsy, reinforcing the value for histopathology for confirmation of diagnosis in treatment studies.

**Other topicals** A range of topical options are purported effective in some sarcoids, however, controlled studies are lacking. These include bloodroot extracts, heavy metal products, and xanthates. European mistletoe (Viscum album aqueous extracts) has been used in human oncology and has proposed cytotoxic and immune-modulating effects. One controlled, blinded study in 53 horses showed 38% complete resolution rate with treatment compared to 13% in the control group.

**What is the prognosis for sarcoid?**
The behavior of sarcoids is unpredictable with even lesions of similar appearance varying notably in progression. Lesions may multiply, sometimes very rapidly, or remain completely static for years. Occasional spontaneous complete and permanent resolution is suggested to occur. In some cases, treatment of one or a few lesions has resulted in clinical improvement of lesions at different sites. Prognosis may be best with few small lesions and in horses less than 4-5 years old. Consideration should be given to potential contagion to other in contact or nearby horses. Limb and periocular lesions, in general, and more aggressive fibroblastic and malevolent forms, in particular, appear to have a poorer prognosis. A poorer prognosis is suggested after unsuccessful attempts at treatment, so initial treatment should ideally be aggressive, sustained, and followed through closely.
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EQUINE SARCOIDOSIS (GENERALIZED GRANULOMATOUS DERMATITIS)

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Introduction
Equine sarcoidosis is a rare disease that is also known as equine generalized granulomatous disease, equine systemic granulomatous disease, equine histiocytic disease and equine histiocytic dermatitis. It is typified by the infiltration of the skin and other organs with macrophages (histiocytes) and multinucleated histiocytic giant cells producing sarcoidal granulomas (accumulation of epithelioid macrophages and multinucleated giant cells).

Etiology
The etiology of this disease is unknown, although the nature of the inflammatory process (granulomatous) suggests that it may be initiated by an infectious agent. Extensive efforts have been made to look for potential infectious agents that might trigger this disease, including Mycobacterium spp, Borrelia burgdorferi, Coccidioides immitus, Cryptococcus neoformans, Corynebacterium pseudotuberculosis and equine herpesvirus 1 and 2. None have been found to date. One horse was noted to be infected with Mycobacterium intracellulare serotype 8.

Signalment
The age range at time of presentation has varied from 3 months to 21 years. In one study of 22 horses, the mean age at presentation was 10 +/- 4.6 years (range 3 – 17 years). Gender predispositions have varied with the study. In two studies, geldings were more commonly affected. In another, mares were more commonly affected. The disease has been noted in warmblood, Friesian, Arabian, standardbred and thoroughbred horses, and ponies. There may be a predisposition in thoroughbred horses. There is no seasonal variation.

Clinical Signs
Clinical signs may develop slowly or rapidly. In general, cutaneous signs include an exfoliative dermatitis and/or nodules. Nodules tend to be much less common. The exfoliative dermatitis is characterized by focal (often well demarcated), multifocal or generalized areas of severe scaling and crustng, and variable alopecia. They may occasionally be painful. Affected skin may feel warm to the touch. Pruritus may be present, but tends to be uncommon. The nodules are dermal to subcutaneous and may or may not be associated with an overlying exfoliative dermatitis. They may be subcutaneous edema associated with the nodules. They may be painful. There may be a peripheral lymphadenopathy. Internal organs may be involved in the same granulomatous process. In decreasing order of incidence, this would include the lungs, lymph nodes, liver, GI tract, spleen, kidneys, bones and central nervous system.

The author favors a categorization of these clinical presentations as described by Sloet.

1. Generalized exfoliative sarcoidosis – widespread exfoliative dermatitis +/- generalized nodules. The exfoliative dermatitis tends to occur on the face, girth area, ventral abdomen, axillary and inguinal region, neck, shoulder, legs, prepuce and scrotum. If not nodular at the time of presentation, nodules may eventually develop. The generalized exfoliative form...
is often associated with a generalized “wasting syndrome” and one or more of the following signs: low grade fever, exercise intolerance, respiratory distress, weight loss and peripheral lymphadenopathy. Other signs that may be encountered are dictated by organ system involvement including diarrhea and icterus with liver and gastrointestinal involvement and lameness with bone lesions. If the “wasting syndrome” is not noted at the time of presentation, it usually will develop with time.

2. Partially generalized sarcoidosis – exfoliative dermatitis and/or subcutaneous nodules on a limited body area (e.g., hind limb and thigh; front limb and shoulder). There may be a peripheral lymphadenopathy. Individuals with this form usually go on to develop a generalized exfoliative dermatitis and systemic involvement.

3. Localized – localized area/areas of exfoliative dermatitis (hyperkeratotic, crusted, alopecic plaques) of the lower limb (below the elbow or knee) that may be variably edematous, painful and occasionally associated with lameness OR one focal area of exfoliative dermatitis in some area of the body. This form is not associated with systemic involvement. This form is only occasionally noted to become generalized.

Clinicopathologic Abnormalities
These are variably seen as a leukocytosis, neutrophilia, mild non regenerative anemia, hyperfibrinogenemia, hypoalbuminemia, hypercalcemia and abnormal liver or kidney function tests.1,7

Differential Diagnoses
Differential diagnoses for the exfoliative dermatitis include dermatophilosis, dermatophytosis, pemphigus foliaceus, erythema multiforme, drug eruption, epitheliotropic lymphoma, exfoliative eosinophilic dermatitis, primary seborrhea, systemic lupus erythematosus and toxicosis associated with hairy vetch, arsenic, iodine, aluminum and silicone.1

Diagnosis
The diagnosis is established by rule out and tissue biopsies. Tissue aspirates of nodules are suggestive in that they reveal granulomatous inflammation. Multiple (at least 3 or more) skin biopsies should be taken from various lesional areas. Hairs may be gently clipped from the affected area, but the skin should not be surgically scrubbed prior to biopsy. If exfoliative lesions exist over a nodule, the biopsy should be deep, obtaining nodular tissue. If there are no skin lesions over a nodular area, this area can be clipped and surgically scrubbed prior to nodule biopsy. Histologic changes include a multifocal to diffuse, dense accumulation of macrophages (histiocytes) that may extend throughout the dermis (granulomatous dermatitis). These are sometimes referred to as “sarcoidal granulomas” because the macrophages are epithelioid in appearance, as is seen with sarcoideal granulomas in people. Granulomatous areas contain characteristic multinucleated giant cells. Smaller numbers of lymphocytes and occasionally plasma cells may be present. Neutrophils tend to be uncommon. A concurrent vasculitis has been noted in the focal form of the distal extremities in two horses.5 Other changes include mild acanthosis, mild hyperkeratosis, mild spongiosis and crusts on the surface of the epidermis that are made up of parakeratosis, serum and degenerating inflammatory and epithelial cells.
Therapy
The mainstay of therapy for this disease has been glucocorticoids. Recommended starting dosages of prednisolone have varied from 1-2 mg/kg PO/day\(^5,6\) to 2-4 mg/kg/day\(^7\) (author starts at 1.5 – 2.0 mg/kg/day). Once significant improvement has been seen in lesional areas (2-4 weeks), the daily dose is gradually tapered over several weeks (e.g. if started at 1.5 mg/kg/day, reduce to 1.0 mg/kg/day for 2 weeks, then 0.5 mg/kg/day for 2-4 weeks, then 0.25 mg/kg/day for 2-4 weeks) then try to switch to every other day therapy at 0.5 mg/kg eod, then very gradually begin to taper this. A recurrence of signs would warrant increasing the dose of steroid to induce remission, followed by a gradual reduction to a higher maintenance dose that appeared to be controlling the problem. Dexamethasone may be used to induce remission and may actually do so more effectively than prednisolone. Starting dosages have been variably given as 0.04 mg/kg – 0.08 mg/kg IM once daily for 7 – 14 days\(^5,6\) to 0.2-0.4 mg/kg/day.\(^7\) The author starts at 0.1 – 0.2 mg/kg/day. After the first 7 -14 days, the dose is halved and given for 7 – 14 days. Once a good response has been noted, it is ideal to switch to oral prednisolone for longer term therapy.

Other therapies have been suggested but are largely adjunctive treatments to provide some steroid sparing effects. These have included omega 3/6 fatty acids given at standard recommended dosages (trial period of at least 3 months)\(^5,6\), methylsulfonylmethane (2 tsp, or 10 – 12 gm BID) or pentoxifylline (8-10 mg/kg BID).\(^5-7\) There is no data to support the efficacy of these therapies. Dietary changes have been suggested because of the similarity of this disease to that associated with hairy vetch toxicity (i.e., discontinue artificial supplements; avoid any potentially toxic plants; return to “natural” feeding patterns) but there is no substantiation of benefit associated with these recommendations.

Prognosis
Although uncommon, spontaneous remissions of both the generalized and local forms have been noted (more likely with the local forms). The prognosis for the generalized and partially generalized forms of the disease is fair to poor. The prognosis appears to be better if there is no obvious systemic involvement. Pulmonary signs have been noted to respond to prednisolone therapy.\(^1\) Gastrointestinal signs appear to be associated with a poor prognosis.\(^1\) In one study, 7 of 7 horses with generalized or partially generalized forms of the disease were poorly responsive to therapy and were euthanized within months.\(^5\) In another study of 6 cases (one with systemic signs), 5 of 6 responded to glucocorticoids.\(^3\) 2 of 6 eventually went in to spontaneous remission. In another study, 5/9 had internal involvement (most commonly pulmonary). 3/9 were euthanized because of a lack of response to therapy. All had systemic involvement. 6/9 responded to glucocorticoids. Follow-up on 3 of the 6 showed spontaneous remission after several months of prednisolone in one case and spontaneous remission with no significant steroid therapy in the other two.\(^1\) The prognosis for the localized form of the disease is “fair”. One study evaluated 9 individuals that were treated with glucocorticoids. Remission was noted in 2 cases. 7 improved, but required indefinite maintenance therapy in order to maintain partial remission.\(^4\) In another study of 14 horses with lesions on the lower limbs, 2 showed poor response to glucocorticoids, 4 spontaneously resolved (one after being treated with glucocorticoids), 8 improved on glucocorticoids, but required daily glucocorticoids to maintain remission (0.2, 0.3, 0.5, 0.5 – 1.0, 1.2 mg/kg/day respectively).\(^5\) These lower limbs remained abnormal to various degrees, but overall were acceptable to owners.
Selected References

There are no written proceedings for this session — please use this space for your own notes.
**APPROACH TO EQUINE PRURITUS**

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**Most cases of pruritus in the horse are due to**

- Ectoparasites: *Culicoides, Chorioptes, Trombiculidae* (‘chiggers’), various biting flies
- Allergies: atopic dermatitis, fly-salivary protein allergies (especially *Culicoides*), contact, food (rare)
- Infections: staphylococcal pyoderma, dermatophytes, *Malassezia*

**Questions to ask when obtaining a history:**

- Does the horse scratch (itch, self-bite, rub or lick) excessively?
- There is a wide range of awareness among horse owners if their horse is actually pruritic. This is usually dependent on how much time during a week the owner spends with the horse.

*Duration/seasonality?* Seasonal pruritus, if warm weather, suggests either atopic dermatitis (pollens) or ectoparasites. Photo-aggravated vasculitis may be worse in the sunnier times of year. If cold weather worsens pruritus, this suggests atopic dermatitis (dusts, molds) or ectoparasites in the barn (*Dermanyssus gallinae*).

*Progression of pruritus?* Always important to know if what is presented is the most pruritic, or if the horse has had worse episodes in the past. If a rash (papules/erythema) is present, did the rash precede the pruritus or was the pruritus the cause of the rash (i.e., is it a rash that itches [ectoparasites, bacterial- or dermatophyte-caused folliculitis] or an itch that rashes [allergies]).

*Previous treatment?* If there was previous treatment, was there improvement? Did it make the pruritus worse? Remember that a statement such as ‘antibiotics (or steroids or shampoos, etc.) did not work’ tells NOTHING – one needs to know the type, dosage, and duration. Ask if the owner has either kept a list or still has all bottles, vials, etc. of previously utilized drugs, including topical medications. Response to antibiotics usually suggests a pyoderma, however, some clients regard all pills as “antibiotics”. In addition, oftentimes antibiotics are given concurrently with corticosteroids and the owner will neglect to mention this fact.

*Environment?* Is the horse mainly kept in a barn, a pasture, have contacts with other horses or other animals?

*Diet?* While food allergies are very rare in herbivores, the list of foodstuffs consumed should include hays, grains, and all supplements. Horses on a poor plane of nutrition may be more susceptible to infectious agents such as pyoderma, dermatophytes and lice. Also, the possibility of ingestion of plants containing potential liver toxins or photodynamic agents should be investigated. The most pruritic horse the author has ever seen was seemingly associated with end-stage liver disease.
Travel? Has the horse traveled out of the immediate area? Race horses and expensive breeding horses may be shipped almost anywhere in the world, and may encounter diseases that are not endemic to the veterinarian’s geographic area.

Other animals or people affected? Involvement of other animals or people obviously points towards contagious diseases (such as dermatophytes). However, lack of disease in other mammals does not necessarily rule this out. Also to be considered are a genetic cause (if horses are related), an environmental factor (ectoparasites, pollens, molds, dust), contaminated food stuffs, or contact allergens or irritants.

Type of lesions

Alopecia usually signifies traumatic hair loss due to pruritus. Examining the distal ends of the hair shafts may confirm this if those ends are frayed or squared off. Alopecia due to metabolic or follicle cycle abnormalities typically has hair shafts that are tapered.

Papules may indicate allergic, infectious, or ectoparasitic etiologies. Examining with a hand lens to determine if individual papules are associated with a hair shaft (indicating a folliculitis [usually dermatophytes or bacterial infection] is helpful).

Pustules usually indicate an infectious etiology, but may rarely be present in pemphigus foliaceus. Cytology can usually determine the difference.

Urticaria may occur with or without pruritus. The most common causes for urticaria in the author’s practice are biting flies, atopic dermatitis (which may or may not be pruritic), and drugs. Less commonly urticaria may be caused by vasculitis (including purpura hemorrhagica), dermatophytes, or pemphigus foliaceus. In the latter two conditions the lesions usually evolve into the more common alopecia or crusts, respectively.

Epidermal collarettes are often over-looked, and are the ‘remains’ of a pustular disease, usually pyoderma; rarely a drug reaction or pemphigus foliaceus.

Distribution of lesions

Ventral Midline Dermatitis (VMD) is most often associated with Culicoides spp (in a diffuse pattern), Haematobia spp (‘horn flies’) in a focal pattern, or rarely, Onchocerca cervicalis or Chorioptes infestation.

Mane and Tail is most often associated with Culicoides spp

Pastern pruritus is often evidence of Chorioptes infestation, but may also be associated with vasculitis and occasionally, bacterial or dermatophyte infection. Rarely, contact allergy or atopic dermatitis may affect this area of the body.

Facial pruritus may be due to atopic dermatitis, black flies (Simulium sp) (especially between rami of mandible), or stable flies (Stomoxys calcitrans) – these may also cause pruritus on the front legs and cranial thorax.
Caudal trunk (‘tail-rubbers’) This presentation may be caused by Culicoides spp, pinworms (Oxyurus spp), atopic dermatitis, yeast (Malassezia spp - these may be found between the mammary glands of mares or in the preputial fossa of males but the pruritus is evident by tail rubbing). Rarely, food allergy may cause this presentation.

Diagnostic Tests
These include skin scraping for ectoparasites, skin scraping for cytology, hair shaft examination, bacterial and/or fungal culture, skin biopsy, and intradermal and/or serologic testing for allergens. These will be discussed in detail in other lectures in the Equine Programme.

Treatment
Definitive treatment of pruritus depends on the underlying cause. For non-specific anti-pruritic treatment, the author uses (when corticosteroids are not contraindicated) prednisolone (1mg/kg q24h, then taper), or dexamethasone (0.05 – 0.01 mg/kg q 24h, then taper). Prednisolone seems to be better absorbed orally in horses than prednisone. The injectable dexamethasone solution may be used orally, although the bioavailability is 60-70% of the injectable route.

Antihistamines are sometimes quite effective: hydroxyzine pamoate (0.4-0.8mg/kg TID or BID ), cetirizine (0.4 mg/kg BID) or doxepin (a tricyclic anti-depressant with antihistaminic effects – 0.6 mg/kg BID). (Cetirizine is the major metabolite of hydroxyzine, and in most countries is more expensive, so the author usually uses hydroxyzine). Antihistamines rarely cause either drowsiness or excitability in horses — the author has seen this in less than 5% of horses receiving these drugs. The commonly used antihistamine pyrilamine maleate [Tri-Hist Granules] has been shown to have poor bioavailability when given orally in the horse, but may be detectable in the urine up to 1 week after a single dose, which may present difficulties with withdrawal times.

Selected References


One trend that is coming to light is the fact that horses, as well as human beings, dogs and cats, commonly have combination allergies (i.e., insect allergies, atopy, recurrent airway obstruction (RAO; equine asthma), drug and food hypersensitivities). It is therefore important to keep in mind key concepts such as “allergic threshold” (level above which allergic symptoms are noted) and “summation of effect” (the sum of antigenic contributions) when treating equine allergic dermatoses. A successful therapeutic protocol must encompass addressing the patient’s predisposing and environmental influences along with treating the secondary perpetuating factors (bacteria and Malassezia), all while specifically targeting the primary etiology. Even if dealing primarily with Culicoides spp hypersensitivity, addressing other components of the patient’s allergies may lower the allergen load, such that the summation of effect is brought below the allergic threshold. A multimodal approach will help to minimize the need and side effects of glucocorticoid therapy, allowing steroids to only be used in bursts of 1-7 days to address allergy flares (Fig. 1). Lastly, client expectations must be set so that allergies are regarded as controllable and not curable, lifelong and not short-term, as well as ever-changing and not stagnant. Equine clinicians must also incorporate client restrictions (e.g., boarding versus horse on farm) when it comes to selecting the most appropriate therapeutic protocol for their allergic patient. Understanding the various treatment options will help get all the right pieces of the therapeutic puzzle together.

**Figure 1.** Multi-modal therapy analogy whereby the “trench diggers” are therapeutic approaches with minimal side effects and the “fire retardant” is glucocorticoids used in bursts to put out fires.
Environmental Control
Regardless of the manifestation of allergic reaction in a horse (cutaneous or respiratory), avoidance or, at a minimum, a reduction in allergen exposure is the best treatment for allergies. Although this option if often impractical, it must be offered as an adjunct to systemic therapy for the patient in lieu of lifelong anti-inflammatory therapy. Recommendations may include the following:

Changing Environments
Moving an affected horse from the current environment to a different part of the country or simply down the road to a different barn style (bank barn versus open air) with altered turn-out procedures depending on allergic reactions; that is, leave the horse on pasture if mold spore and dust allergies are the primary issue or keep the horse indoors especially in the later afternoon if the patient has summer pasture associated allergies.

Bedding
Minimize dust exposure in the barn by switching to rubber mats and/or minimum dust-generating bedding. Use of pelleted rations, grass silage, hydroponic or wet down hay all help to reduce food-based dust exposure. An investigation of various peat moss composites revealed fungi in sphagnum peat, various levels of endotoxin pending storage conditions, and the presence of thermophilic actinomycetes and Aspergillus fumigatus in few-flowered peat materials. The concentrations of inhalable dust were smaller in the few-flowered peats than in the sphagnum peats. It was concluded that there are differences in the dustiness and hygiene quality of peat bedding. Shredded cardboard or shredded flax as an alternate source of minimal dust-generating bedding when used in conjunction with low-dust forage, might be appropriate in the provision of minimum-dust management for allergic horses.

Insect Control
Insect control in the environment can be accomplished by moving affected horses away from standing water, manure piles, compost piles, and sheep or cattle. Culicoides are most active at sunset, less so at sunrise and very few or no midges are present in the environment in the afternoon or at night. Based on these observations, turn-out in the evening or afternoon and stabling before dusk to after dawn may be simple management procedures to decrease the patient’s exposure to Culicoides. In barns without stable managers, this may be difficult to incorporate into the establishment’s daily routine. Other alternatives include the use of flysheets and/or fly masks impregnated with permethrin repellant, box fans to circulate air within the stall decreasing the ability of the Culicoides to land on the patient, timed-released insecticide sprays within barns, using fly wasps to address insects commonly found in compost and manure, and fish in ponds to ingest insect ova and larvae.

Dietary Management
Food allergens are another route by which clinicians can help minimize/eliminate allergen load by avoidance alone. Dietary trials to diagnose food hypersensitivity or intolerance in horses currently consists of a 4 to 6 week trial employing novel feed sources such as timothy, rolled oats or barley if not routinely fed. As alfalfa is commonly incorporated in various treats, supplements, and hay cubes, it is often implicated as a top allergen in food allergic horses. Similarly, molasses, most likely because of its prevalence in feed and treats, is another component to avoid during a
dietary trial. Discontinue any unnecessary supplements, vitamins, and other drugs as they may result in non-IgE mediated reactions. To confirm food involvement in the allergic symptoms, a dietary challenge is performed by reintroducing one item every 7 days and examining the patient for any evidence of exacerbation of clinical signs. Unlike canine and feline patients, the food trial guidelines have been based on anecdotal information and not controlled studies. Therefore, the length of the trial and challenges in equine medicine should be based on clinical response or lack thereof.

Topical Therapy
When treating horses with allergies, fly control is a mandatory part of any therapeutic regimen. A spray containing 2% permethrin has been shown to be effective in treating horses with Culicoides spp hypersensitivity. A study evaluating a 3.6% pour-on concentrated permethrin did not provide statistically significant protection compared to non-treated horses. However, the author uses a topical 44% concentrated permethrin canine product off-label in horses. Vitamin E is applied at the site of application (typically under the mane) to prevent permethrin-induced paresthesia. Other recommended repellants include various over-the-counter bath oils diluted 50:50 with water; bug guard lotions with sunscreen; or an aqueous N,N-diethyl-m-toluamide (DEET) solution at a concentration of 16.6%. Application frequency will depend on the product selection, geographic insect distribution, season of the year, and severity of the patient’s condition.

Shampoo therapy should not be overlooked in the treatment of equine allergies. The simple act of bathing with COOL water has several benefits: 1) rehydrates the skin improving the integrity of the epidermal barrier; 2) results in vasoconstriction hence decreasing delivery of inflammatory mediators to the skin; 3) helps to minimize percutaneous absorption of allergens by washing them off; and 4) with appropriate ingredient selection, addresses secondary superficial infections. The selection of shampoos should be based on the patient’s skin condition and may include colloidal oatmeal products (shampoos, conditioners and bath treatments) with or without a local anesthetic (pramoxine HCl) or corticosteroids for pruritic dermatoses; sulfur/salicylic acid shampoos for horses with excess scale; antimicrobial shampoos containing accelerated hydrogen peroxide, benzoyl peroxide, chlorhexidine or imidazoles if secondary infections have been identified; or a combination of one or more of the above.

Lime sulfur is still a very effective multimodal topical therapeutic as it provides not only ectoparasitic activity, but also antipruritic, antiseborrheic and antimicrobial effects. Although off-label, it is a safe and proven treatment option that can be applied as a dip or spray on horses.

Topical steroids have also shown good efficacy when treating small animal patients. Unfortunately, most of these products are not labeled for use in horses. Several topical steroid products that I have used for treatment of localized lesions include:
1. A mild 1% hydrocortisone, leave-on conditioner in a non-irritating base
2. Steroid ointments or creams containing alclometasone 0.05% or mometasone 0.1% with mild-moderate and high potencies, respectively
3. A 0.015% triamcinolone spray
4. An esterified 0.584 mg/ml hydrocortisone aceponate spray
5. An otic product containing 0.1% mometasone, gentamicin and clotrimazole
When choosing a topical steroid, one must strive for products with minimal side effects; that is, minimal to no hematological and biochemical changes, suppression of the adrenal axis, and local cutaneous alterations (atrophy, alopecia, comedone formation, and secondary infections).

**Systemic Therapy**
Along with the traditional IgE-mediated allergic reactions, it appears that the T-helper-1/T-helper-2 paradigm, along with all its cytokine alterations, exists in horses with recurrent airway obstruction and most likely cutaneous allergies. Similar to other domestic species, the focus on treatment of allergies should therefore be directed at re-establishing the balance of T-cell interactions, hence minimizing the production of IL-4, IL-5 and other inflammatory mediators such as chemokines, and the traditional products of IgE-mediated mast cell degranulation.

*Allergen Specific Immunotherapy (ASIT)*
Greater details regarding ASIT in horses are covered in other notes. The following are benefits of allergen specific immunotherapy:

1. Positive responses ranging between 60 to 80% efficacy
2. Minimal side effects (e.g., local injection reaction)
3. Decreased dosing frequency/workload for the owner (injections q 7-28 days)
4. Cost effective because it is a weight-independent therapy
5. Not a banned treatment by Fédération Equestre Internationale (FEI) and therefore can be used throughout show events, when allergies often affect patients
6. Unlike other therapies that provide symptomatic temporary relief, ASIT may achieve a cure/remission

Based on the above, ASIT in horses should be considered a viable therapeutic modality for long-term control of insect hypersensitivity, recurrent urticaria/pruritus and recurrent airway obstruction. ASIT provides an alternative treatment modality that may allow the horse to return to performance without negative consequences while not compromising the rider’s ethics.

*Polyunsaturated N-3 And N-6 Fatty Acids (PUFAS)*
Most mammalian cell membranes incorporate PUFAs, and they are thought to create a shift in the production of pro-inflammatory mediators to non- or anti-inflammatory mediators in the arachidonic acid cascade. Other possible mechanisms by which PUFAs exert their positive clinical benefit in atopic dermatitis are still under investigation, but include repair of the epidermal barrier when using omega-6 fatty acids. Fatty acid supplements have shown variable reported responses in horses. The difference in results is most likely attributable to the variability of the research parameters, namely:

1. Source and dose of fatty acid being given and in food - linseed oil, flaxseed meal vs. oil, marine fish oils
2. Type of allergic reaction being evaluated - insect allergy versus atopy versus other
3. Parameters being evaluated - local intradermal test reaction versus circulating plasma fatty acid or inflammatory mediator concentrations
4. Length of the study
5. Number of horses in the study
6. Study design (e.g., randomized double-blinded placebo controlled +/- crossover with washout)
7. Geographic location of the studies - Florida, Oregon, United Kingdom, Canada

Hence, to make any conclusions on the efficacy of the essential fatty acids based on current equine studies is difficult. Our knowledge of clinical benefits of PUFAs in recent canine atopic dermatitis studies along with the lack of significant adverse reactions (mainly diarrhea), would justify its use in equine dermatology as adjunct to any long-term anti-inflammatory protocol. Typically, improvement in pruritus and/or skin condition should be noted within 2 to 8 weeks after initiating therapy. A variety of PUFAs exist on the veterinary market and are typically administered at their labeled dose.

Epidermal barrier repair

Epidermal barrier repair products are used to repair seborrheic skin conditions and maintain an intact epidermal barrier to minimize percutaneous absorption of allergens and adherence of microbes. If shampoo hydrotherapy is possible, appropriate selection of shampoos based on active ingredients used with COOL water on a weekly basis will help to minimize surface microbes, wash off accumulated allergens, provide anti-inflammatory relief and help repair the epidermal barrier.

Topically applied barrier repair products may contain combinations of sphingosine, ceramides and free fatty acids all of which are essential building blocks for the corneal lipid envelope to help rebuild the epidermal barrier. Other products contain essential fatty acids, essential oils, humectants, emollients, and lubricants that help to fill in the gaps in a damaged skin barrier. Because of the minimal incidence of side effects, barrier repair products should be incorporated into the multimodal approach of both treatment and prevention of allergic symptoms.

Antihistamines and Tricyclic Antidepressants

Antihistamines and tricyclic antidepressants (TCA) provide a non-steroidal alternative for long-term control of allergic reactions in horses. The H1 receptor antagonist activity of these drugs is sometimes complemented by other mechanisms of action including anti-serotonin/serotonin re-uptake inhibition. Exact dosing and recent pharmacokinetic studies are emerging in the horse. The following are the antihistamines and TCAs that are being prescribed to horses (in my personal order of preference):

1. Cetirizine 0.2-0.4 mg/kg BID (57-79% inhibition of wheal formation after 7 days)
2. Hydroxyzine hydrochloride or pamoate 0.5-1.0 mg/kg TID (urticaria>>pruritus)
3. Doxepin hydrochloride 0.5 - 0.75 mg/kg BID
4. Amitriptyline 1-2 mg/kg BID
5. Chlorpheniramine 0.25 mg/kg BID
6. Diphenhydramine 0.75-1 mg/kg BID
7. Fexofenadine 10mg/kg TID
8. Pyrilamine maleate 1 mg/kg BID

Similar to human beings and other domestic animal species, there is tremendous variation in response to antihistamines/TCAs. It is sometimes necessary to try several different classes of antihistamines at 2-week intervals before finding the most effective option. Despite the paucity of documented synergism between antihistamines/TCAs and other anti-inflammatory therapies in
the horse, it is worthwhile to combine therapies based on the numerous positive studies in dogs and cats.

Although antihistamines and TCAs have fewer reported side effects (light sedation, occasional personality changes) than corticosteroids, one must always keep in mind the anticholinergic properties of these medications in patients with glaucoma, gastrointestinal atony, cardiac arrhythmias, or urinary retention problems. Advise owners to contact show authorities regarding drug restrictions/withdrawals at least 14 days prior to the event.

**Phosphodiesterase Inhibitors**

Pentoxifylline (PTX) is a synthetic xanthine derivative related to caffeine and theophylline. Its phosphodiesterase inhibition imparts 3 major therapeutic benefits:

1. Improves wound healing and connective tissue disorders:
   a. Increased fibroblast collagenases
   b. Decreased fibroblast collagen, fibroblast fibronectin, fibroblast glycosaminoglycans
   c. Decreased response to Tumor Necrosis Factor (TNF)-alpha

2. Rheologic agent:
   a. Decreased platelet aggregation and adhesion
   b. Increased red cell deformability
   c. Decreased vasoconstriction
   d. Increased plasminogen activator, plasmin, antithrombin III
   e. Decreased fibrinogen, α2-antiplasmin, α1-antitrypsin, and α2-macroglobulin effects

3. Immunomodulator:
   a. Inhibition of T- and B-cell activation and proliferation
   b. Increased leukocyte deformability, chemotaxis
   c. Decreased leukocyte adhesion and aggregation
   d. Decreased neutrophil superoxide release, neutrophil degranulation
   e. Decreased monocyte TNF-alpha production, leukocyte response to TNF-alpha, lymphotoxin, interferon-gamma
   f. Decreased production and leukocyte response to IL-1 and IL-12
   g. Increased production of IL-10 and PGE2
   h. Decreased natural killer cell activity

By one or many of the mechanisms above, PTX potentiates the effectiveness of many medications including steroids (steroid-sparing effect). For this reason, and PTX’s rheologic activity potentially minimizing the risk of laminitis, the author tends to use pentoxifylline (8-10 mg/kg PO BID to TID) in conjunction with steroids, thereby providing a non-steroidal alternative with minimal side effects (hyperexcitability, sweats) for the purpose of tapering or eliminating the need for glucocorticoids in immune-mediated and allergic dermatoses. Response rates can be quite variable due to the poor bioavailability of oral pentoxifylline. Considering the increased rheologic activity, this medication should be used with caution in conjunction with anticoagulants or in patients with hemorrhagic disorders.
Corticosteroids
Corticosteroids have long been a standard therapy for allergies in the horse. Corticosteroids work primarily by gene repression and inhibition of nuclear factor-kappa B, which directly or indirectly prevents the production of cytokines, chemokines, cell adhesion molecules, complement factors, and prostaglandin and leukotriene synthesis involved in the allergic response. Individual sensitivity to glucocorticoids may be directly related to Type 1: Type 2 11-β-hydroxysteroid dehydrogenase ratio. Judicious use, appropriate amounts and intervals are key to minimizing adverse reactions.

The following are the two most commonly used glucocorticoids used for the short-term treatment of equine allergies: 1) Prednisolone available as tablets or a compounded syrup or powder is typically dosed at 0.5 - 1.5 mg/kg/day for 7-14 days during the induction phase then tapered to 0.2 - 0.5 mg/kg every 48 hours over 2-5 weeks for maintenance. If cost is an issue, prednisone may be substituted for prednisolone; however, prednisolone has been shown to have greater bioavailability in horses; 2) Dexamethasone available as a powder, tablets, injectable (per os or IV); injectable dexamethasone solution given orally is 60-70% bioavailable compared to the IV route. The initial loading oral or IV pulse dexamethasone dose is 0.05-0.1 mg/kg daily for 3-7 days, then tapered to 0.01-0.02 mg/kg every 48-72 hours for maintenance. This regime is particularly helpful in more refractory cases.

Ciclosporine and Oclacitinib
Ciclosporine, a calcineurin inhibitor, has been used in the management of human, feline and canine atopic dermatitis, and most recently, in a miniature donkey. Oclacitinib is licensed for use in dogs, with off-label reports of its use in cats. However, the lack of pharmacokinetic data in horses and the cost of both of these weight-dependent dosed medications limits their use in equine medicine at this time.

Other Treatment Options
Methylsulfonylmethane can be used in conjunction with other anti-inflammatory therapies for its antioxidant properties. Controlled studies are lacking regarding its efficacy in equine allergies; however, due to the absence of significant side effects, the author continues to use the product initially at 10-12 gm/500kg BID, then taper to a once daily dose.

With the future of gene microarray analysis of cytokine release in allergic horses, more individual and directed therapeutic recommendations may be on the horizon such as receptor antagonists (Platelet Activating Factor receptor antagonist, eotaxin receptor (CCR3) antagonists); enzyme inhibitors (protein kinase C); and species-specific monoclonal antibodies directed against key cytokines (Anti-interleukin-4 monoclonal antibody (pascolizumab), anti-interleukin-31 monoclonal antibodies). As well, the use of potentiated or conjugated immunotherapy using bacterial CpG motifs or cell wall extracts, lysosomes, or other proteins to help potentiate the immune system’s recognition of the offending allergens within immunotherapy ultimately allow us to turn off the allergic response with minimal side effects and cost.
Key Points:

1. Atopic dermatitis/environmental allergies can cause pruritic dermatitis, urticarial to angioedematous reactions, as well as, allergen exacerbated recurrent airway disease in horses.
2. Insect hypersensitivity reactions are generated by not only the bite and response to salivary antigens of the insect in question, but also from inhalation or percutaneous absorption of the desiccated insect parts.
3. As local amplification of IgE occurs in response to percutaneous absorption of allergens, intradermal allergy testing still provides the most accurate assessment of the local immune response. In correlation with the historical data, the results of the intradermal allergy test are used to select antigens to be incorporated into the allergen-specific immunotherapy treatment set.
4. Treatment of equine allergies requires a multi-modal approach including environmental control, topical and systemic therapy, and allergen specific immunotherapy.
5. Allergen specific immunotherapy should be considered for all presentations of equine allergies including hives, heaves, scratches, and head-shaking as it is a cost-effective weight independent treatment option that is not banned by the FEI, has minimal adverse effects and may achieve a cure.

Selected References
ALLERGEN TESTING AND IMMUNOTHERAPY IN THE HORSE

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Introduction
In contrast to the dog, respiratory allergies play a much bigger role in the horse, although cutaneous manifestations of hypersensitivities are also well recognized in the equine species. Positive correlations between symptom severity and exogenous factors such as seasonal pollen counts were observed. Exposure to moldy hay is considered most important in equine recurrent airway obstruction (RAO). Challenge with moldy hay or mold extracts led to exacerbation of clinical symptoms and basophil histamine release or histamine release by pulmonary mast cells in response to stimulation with fungal allergens or hay extract was higher in RAO-affected horses than in healthy controls. IgE antibodies specific for crude extracts of *Aspergillus fumigatus*, *Alternaria alternata*, *Penicillium notatum*, and the recombinant allergens Asp f 7,8, 9 and Alt a 1 in BAL or serum were detected in horses with RAO. IgE antibodies specific for crude mold extracts were not different between healthy and affected horses. In contrast, IgE and IgG against recombinant allergens was detectable more frequently in RAO affected horses than in healthy controls. Symptomatic therapies with antihistamines, glucocorticoids and fatty acids have been described as variably efficacious. Glucocorticoids and many antihistamines are not permitted in equestrian sports in many countries and fatty acids are not very effective. Thus there is a great need for specific therapy. For allergen immunotherapy, offending allergens are identified with intradermal tests, serum tests for allergen-specific IgE and basophil degranulation tests. Here the current data on allergy testing and immunotherapy in the horse are summarized.

Intradermal Testing
As mentioned above, horses develop respiratory and skin diseases due to pollen allergens and show positive intradermal test results against tree, grass and weed pollens and mold spores as well as pollen-specific serum IgE. Skin testing used to be the test of choice at least for atopic dermatitis in horses, but in recent years serum testing for allergen-specific IgE or basophil degranulation tests have gained importance in many countries. The allergens used in the test should be chosen according to their importance in the respective environment. Following their injection, mast cell degranulation causes an inflammatory reaction observed as an erythematos wheal. The reactions are graded from 0 (negative) to 4 (strongest positive reaction) considering size, erythema, elevation and induration and the negative control (usually saline or allergen diluent) and positive control (usually histamine 1:10,000 or 1:100,000) facilitate the evaluation. The concentration of skin testing extracts in the horse should be adapted to the species, a higher concentration than recommended in the past was indicated in a study for pollens, the concentrations of insect allergens needed to be diluted more than previously recommended. A positive skin test result indicates only the presence of specific IgE on mast cells in this patient and has to be interpreted in light of the patient's history and environment. If one cannot perform at least 1-2 skin tests per week, evaluation of weak reactions may not be very reliable. In addition, allergens (particularly pollen allergens) have a very short half-life (approximately 2-6 weeks), once diluted to the concentration used for skin testing. Thus, skin testing is financially
viable usually only if skin tests are regularly performed and thus allow efficient usage of the
diluted allergens. False positive reactions are fairly common in the horse, although an increased
number of positive reactions to airborne allergens in horses affected with allergic skin or
respiratory disease points to involvement of IgE. Ideally antihistamines should be discontinued
one week prior to skin testing, and glucocorticoids 2 weeks prior.9

Skin testing may be conducted without sedation in placid horses, however, it may be needed in
other horses. We usually clip an area on the lateral neck to conduct our testing. In horses, test
results should be evaluated after 30 minutes and then hourly until six hours after the allergens
were injected. Most positive reactions were seen after 30 minutes in one study and only 0.5% of
reactions seen at 24 hours were not positive at 30 minutes or 4 hours,10 indicating that reading
intradermal tests for 4 hours should identify most if not all relevant allergens for an individual
patient.

Allergen-Specific Serum IgE
Certainly the big advantage of serum tests is the convenience. Clients do not need to drive to a
specialist, there are no waiting periods. The horse does not have to undergo sedation and does
not need to be clipped in the area where it is tested. Glucocorticoid administration, particularly
long term, may influence serum testing thus withdrawing those is recommended where possible.
Sometimes, grouped allergen testing is utilized for financial reasons and due to the high cross-
reactivity in human medicine. If group testing is positive, then one, some or all of the allergens in
that group may have contributed to the hypersensitivity and thus the clinical signs. If all the
allergens in that group are included in the allergy shots, immunotherapy with nonrelevant
allergens may lead to development of new allergies in addition to treating the present ones. Thus,
immunotherapy based on testing for grouped allergens is strongly discouraged! Correlation
between serum testing and intradermal testing varies. In one study, overall agreement between an
FcepsilonRIalpha-based ELISA and the intradermal test was fair and best of three serum tests
evaluated,11 in another study a histamine release test through basophils was the most accurate test
in determining a Culicoides hypersensitivity in horses.7 However, good studies evaluating
reproducibility of those commercially available tests in the equine species have not been
published, to the author's knowledge.

Allergen Immunotherapy (AIT)
AIT (also named allergen-specific immunotherapy, ASIT), desensitization or hypersensitization
consists of an induction course of increasing doses of allergen extract followed by a maintenance
phase, where a high dose is administered at set intervals, often for several years. In human
medicine, this therapy was studied extensively for patients with asthma and rhinitis. A recent
Cochrane review has concluded that “immunotherapy for seasonal allergic rhinitis results in
significant reductions in symptom scores and medication use and has a relatively low risk of
severe adverse events”. A review evaluating immunotherapy for human asthma came to similar
conclusions. In contrast, studies evaluating immunotherapy for human atopic dermatitis are less
frequently published. In veterinary medicine, double-blinded studies evaluating immunotherapy
are rare and mostly performed in dogs. Most immunotherapy studies are retrospective and thus
more difficult to interpret, as definitions for improvement as well as length of therapy are
different in different studies. Studies and their results were summarized in a recent review.12 In
horses, little is known beyond anecdotal evidence given in conference proceedings and
textbooks. Overall, the success rate quoted in the various sources ranges from 60 to 70% of good and excellent responses to immunotherapy for cutaneous hypersensitivities to environmental allergens.

Immunotherapy for *Culicoides* hypersensitivity in the horse has been not very successful in the past in some studies, in others there was a good success rate with subsequent deterioration after cessation of AIT. No significant added benefit was seen, when AIT was added to insecticidal agents with 5% cypermethrin in one study. Of 54 owners of atopic horses undergoing immunotherapy surveyed with a questionnaire in a retrospective study, 32 owners responded and 27 (84%) reported a clinical benefit. New venues currently explored are immunotherapy of horses with *Culicoides* hypersensitivity with recombinant allergens and non-specific immunomodulation of horses with recurrent airway obstruction with oligodesoxynucleotides.

Selected References


EC TOPARASITES: DIAGNOSIS AND MANAGEMENT

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1. Introduction
Ectoparasites play an important role in equine skin diseases, particularly in warm and humid climates. In many cases the disease caused by the parasites is amplified by the immunologic response built by the host. The primary ectoparasites causing skin disease in horses are insects such as Culicoides, houseflies, stable flies, mosquitoes, and, to a lesser extent, horse and deer flies. Ticks, lice and mites are also common ectoparasites in horses.

2. Insects
A. Culicoides
Culicoides are very small biting flies that are particularly active from dusk to dawn and breed in standing water such as ponds and lakes. They are poor fliers, flying only for short distances and not against the wind. More than 1,000 Culicoides species have been described and in warm humid regions more than 30-40 species may be active at one time. The abundance of Culicoides species is responsible for the variety of clinical syndromes associated with this disease, as different species have different preferred feeding sites. Culicoides spp. bites and the resulting hypersensitivity are a common cause of ventral midline dermatitis in horses. It is common to have more than just one species of Culicoides feeding on one horse and, depending on the species involved, the distribution of the lesions can be primarily ventral or can be more generalized to include the lower limbs, dorsal areas, ears, face, neck, and rump. Culicoides hypersensitivity is considered a mix of both type I and type IV hypersensitivity reactions against several antigens in Culicoides saliva. The lesions consist of papules that crust over and can induce severe pruritus and frequently lead to secondary bacterial infections. Culicoides hypersensitivity is considered one of the most common causes of severe pruritus in horses. Besides inducing hypersensitivity, Culicoides spp. transmit many diseases, including but not limited to Onchocerca, bluetongue virus, and African horse sickness. Diagnosis of Culicoides hypersensitivity is made on the basis of clinical signs, history (in most regions this is a seasonal dermatitis seen only in the warmer months), life style (horse out on pasture at peak feeding times in paddocks close to standing water) and the lack of consistent use of fly repellents. Allergy testing can be considered to confirm a clinical suspicion, but it is important to note that normal horses may also show positive results on both intradermal and serology testing. Thus, the detection of allergen specific IgE indicates exposure and development of IgE but does not necessarily confirms causation by Culicoides. Conversely, some allergic horses may have a negative immediate reaction to intradermal injection of Culicoides allergen. Such horses may only have a type IV hypersensitivity, which will only be evident 24-48 hours after the test. For these reasons, the results of allergy testing must be interpreted in conjunction with the history and the clinical signs. The ultimate diagnosis relies on resolution or decrease of clinical signs in response to aggressive insect control.
Treatment of dermatitis caused by Culicoides involves use of fly repellants to prevent additional bites and reduction of inflammation by use of either topical or systemic glucocorticoids, depending on the severity of the inflammation. Although many products on the market are labeled as fly repellents, the majority are insecticides and not true repellents. True repellent activity against biting insects necessitates high concentrations of permethrin, which is crucial to provide relief to hypersensitive horses. Many spot-on formulations containing 44%-64% permethrin, which provides good repellent activity, are available specifically for use in horses. These products can be used on specific problem areas once weekly, while sprays with lower concentrations (2% permethrin) may be used to cover the rest of the body. Sprays should be used daily for maximum protection, particularly in hot and humid climates, as the efficacy is decreased by exposure to rain and heavy sweating. Other synthetic pyrethroids such as cypermethrin containing products can be effective repellents provided that they are applied daily. In order to minimize bites, it is helpful to move horses to paddocks further away from standing water and to keep horses in the barn in front of fans during peak insect feeding times. These measures help to minimize exposure to Culicoides given the fact that Culicoides fly for short distances and cannot fly against the wind. Fly masks and fly sheets may be used as long as they are changed frequently and kept clean and dry. Incorrect use and maintenance of these items that can trap moisture in the heat of the summer may predispose horses to secondary infections. Because many Culicoides-hypersensitive horses develop a secondary bacterial infection that significantly adds to pruritus severity, antimicrobial therapy is needed in most cases. In mild cases, this can be accomplished by use of topical therapy, such as benzoyl peroxide or chlorhexidine shampoo (weekly), or topical application of oxychlorine-based sprays (daily). In more severe cases, oral antimicrobial therapy may be needed. A good choice is use of an oral potentiated sulfonamide for a minimum of 2 weeks. Because antimicrobial resistance is a growing concern in medicine, topical therapy should be tried first rather than administering systemic antimicrobials in all cases.

B. Horn flies
Horn flies such as *Haematobia irritans* can cause a seasonal ventral midline dermatosis. Horn flies are blood-sucking insects that lay their eggs on cow manure. They typically prefer to settle on the backs of cattle during the cooler parts of the day and on the belly during the hotter part of the day. This fly is not able to complete its cycle if the eggs are laid on horse manure, and this form of dermatosis in horses requires proximity to cows and cow manure. The dermatitis is caused by the fly bites and is characterized by pruritic or painful papules that crust over and leave distinct ulcers and crusts. With chronicity, lichenification and depigmentation develop. It is common to have multiple horses affected in the same herd. Some horse exhibit intense pruritus which leads to self-trauma and secondary skin infections. Diagnosis is based on the clinical presentation and the identification of the flies. The latter rarely leave the host so should be easily detected on the horse. Treatment involves fly control, by both removal of the cow manure and use of fly sprays, and control of the inflammation and any secondary infection. To control pruritus and inflammation, topical glucocorticoids can be used; in more severe cases, a short course of systemic glucocorticoid may be needed. In terms of topical glucocorticoids, a frequent choice is the use of topical triamcinolone. This type of product is easy to use and can minimize the need for systemic therapy, thus decreasing the risk of adverse effects related to systemic glucocorticoid administration.
Other flies that can cause ventral dermatitis (Table 1) include black flies (*Simulium*), horse flies (Tabanids such as *Tabanus* spp, *Chryssops* and *Haematopota*), and stable flies (*Stomoxys calcitrans*). Black flies lay their eggs in running water. Adults are most active in the morning and in the evening and can fly a long distance. Black flies can cause painful bites on areas with little hair. For this reason the ventral abdomen can be a targeted area where bites result in hives and hemorrhagic lesions.

Horse flies lay their eggs on vegetation close to water and can live for several months. They are very aggressive biters and can induce painful bites that are preferentially directed toward the ventral abdomen. Once the bite has occurred, pruritus ensues, leading to self-trauma.

Stable flies lay their eggs on wet shavings and manure. The adults cause pruritic papules that develop a central crust. Repeated bites lead to the development of a hypersensitivity reaction.

Daily application of fly repellent and removal of potential breeding grounds are essential components of the control of these flying insects, regardless of the species.

### Table 1. Summary of flying insects that can cause ventral dermatitis in horses

<table>
<thead>
<tr>
<th>Common names</th>
<th>Species</th>
<th>Conditions for eggs to be laid</th>
<th>Feeding times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midge, biting gnats</td>
<td>Culicoides spp.</td>
<td>Poor flier, only for short distances, not against the wind</td>
<td>Standing water</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Most active from dusk to dawn</td>
<td></td>
</tr>
<tr>
<td>Horn flies</td>
<td>Haematobia irritans</td>
<td>Spends all day on animal (back of cows on cooler times, ventral abdomen on hotter times)</td>
<td>Cow manure</td>
</tr>
<tr>
<td>Black flies</td>
<td>Simulium spp.</td>
<td>Travel very long distance to feed (&gt;10km)</td>
<td>Running water</td>
</tr>
<tr>
<td>Horse flies, deer flies,</td>
<td>Tabanus,</td>
<td>Life cycle can be protracted up to 10 months, strong fliers</td>
<td>Vegetation close to water</td>
</tr>
<tr>
<td>yellow flies</td>
<td>Chryssops,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Haematopota</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stable flies</td>
<td>Stomoxys</td>
<td>Strong fliers</td>
<td>Wet bedding, manure</td>
</tr>
<tr>
<td></td>
<td>calcitrans</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Mites

Four genera of mites can cause pruritic skin disease in horses: *Chorioptes*, *Sarcoptes*, and *Psoroptes*, *Demodex*. The most commonly diagnosed is *Chorioptes*, which should be considered in cases of pastern dermatitis, particularly in draft horses with thick feathers. Mites are minute acarids that live on or in the skin of the host animal. As mites feed, multiply and die, they injure the host's skin. Lesions may be due to mechanical damage, secretion of irritant substances by the mite, an immunologic hypersensitivity to the foreign antigens of the mite or secondary infection. Life cycles are generally 14-28 days long (depending on conditions) and the majority of time is spent on the host. Pruritus damages the skin which promotes the development of bacterial infection. Transmission is by direct contact; occasionally from environment or fomites.

The primary clinical lesion is a papule. The location of the lesions is usually characteristic for the particular species of mite, especially in the early stages. The extent of the lesions depends on the number of mites, their reproductive activity, and the host's reaction. Pruritus is usually moderate.
to severe although subclinical carriers do exist. Self-trauma leads to alopecia, secondary bacterial infections, lichenification, and hyperpigmentation. Chronic infections lead to debilitation, weight loss, stunted weight gain and growth, reduced feed conversion, damage to hide.

Diagnosis is based on history, clinical signs, skin scrapings (1-2 deep scrapings from affected areas and numerous, broad superficial scrapings). Scraping large quantities of scale and crust into a petri dish with mineral oil, and examination under a dissecting scope may facilitate finding mites. A technique to concentrate mites may be employed: collect several crusts and scales, place them in a 10% solution of KOH for 20 to 30 minutes, then centrifuge and examine the sediment. Alternately, a fecal flotation solution can be added to the test tube and a coverslip placed on the top as for a fecal flotation.

Differential diagnoses include:
- Pediculosis (lice)
- Insect hypersensitivities
- Allergy- atopy, contact allergy, food allergy
- Onchocerciasis
- Rhabditic dermatitis
- Auto-immune- pemphigus foliaceus

As part of the treatment it is important to quarantine affected animals and treat the environment with a cleaning agent such as bleach. Include all fomites, stalls, pastures. Treat the affected and all in contact animals with systemic ivermectin (10% solution): 300 mcg/kg PO weekly for 6 doses. Topical products that have shown to be effective include: Selsun Blue® (selenium sulfide) shampoo followed by 2% lime sulfur solution (Lym Dyp®) dips (6 ounces of lime dip/1 gallon water), sponged on every 5-7 days for 6 weeks. Other topical options include: Fipronil spray (0.25%) has been shown to be effective against Chorioptes bovis. These mites can live off the host up to 70 days, so environmental decontamination is imperative, including barn, stalls and bedding, tack and grooming equipment. 

A. Sarcoptic mange
   i) Etiology: Sarcoptes scabiei var. equi in the horse. The female mite burrows in the superficial layers of the skin and feeds on tissue fluids. It is a common cause of pruritic dermatitis in swine, cattle, goats, and, rarely, sheep and horses. This mite is zoonotic and reportable. Sarcoptic mange is an extremely rare cause of dermatitis in horses and has been eradicated in the US for many years. Sarcoptes scabiei spp. can affect a variety of hosts thus cross-infestations between different species and humans are possible.
   ii) Clinical signs: non follicular papules, crusts, excoriations, alopecia, and lichenification. The pruritus is caused by the mite itself and is aggravated by the allergic response that is developed against the mite. Intense pruritus leads to self-trauma and the possibility for development of secondary bacterial infections. Lesions begin on head and neck and progress to entire body.
   iii) Diagnosis: Differential diagnoses for this presentation include allergies particularly atopic dermatitis and Culicoides hypersensitivity with secondary bacterial infections. Additionally, dermatophytosis, dermatophilosis and contact allergy should be considered.
as causes for a pruritic papular dermatitis. Final diagnosis is made by finding the mites on superficial skin scrapings. Since the mites are very difficult to find on skin scrapings, treatment with ivermectin or lime sulfur should be done regardless of the findings of the skin scraping. Mites may be difficult to find; negative superficial scrapes do not rule out sarcoptic mange. The ears are often the best site to scrape.

iv) Treatment: systemic ivermectin or topical lime sulfur dip.

B. Psoroptic mange
Psoroptic mange can present with generalized dermatitis and otitis and can be caused by 2 different mites

*Psoroptes equi*

i) Etiology: *Psoroptes equi* causes the “body mange”. These mites have a life cycle of approximately 2 weeks and can survive off the host for several days. The mites do not burrow; they live on the skin surface or under crusts and scale. This is a REPORTABLE DISEASE IN HORSES (ear mites not reportable). No cases have been reported in the USA since 1970, but sheep scab is still present in many countries, including some in Western Europe.

ii) Clinical signs: primarily pruritus, papules and alopecia. Scaling and crusting develop over time giving the clinical presentation of seborrhea. Distribution of lesions includes the head, base of the mane, and tail base. Clinical signs are more severe in fall and winter.

iii) Diagnosis comes from the detection of the mites on skin scrapings but the mites are difficult to find, therefore, treatment should be initiated if mange is suspected even if the skin scrapings are negative.

iv) Treatment: topical miticidal solutions, in association with whole body dipping. Systemic ivermectin is very effective. *Psoroptes* spp. can survive up to a couple of weeks in the environment thus the transmission may be by direct contact or through an infested environment. Ivermectin (0.3mg/kg PO) is very effective. Treatment should be repeated every 2 weeks for 3 times. Topical eprinomectin pour-on solution (at a dose of 0.5mg/kg body weight weekly once for four applications) has also been reported to be an effective treatment for Psoroptic mange.

*Psoroptes cuniculi*

i) Etiology: *Psoroptes cuniculi* causes otitis.

ii) Clinical signs: ear rubbing and head shaking; thick greasy crusts build up in the ear canal.

iii) Distribution: external ear canal, occasionally spreads to the face, neck and body.

iii) Treatment: Systemic ivermectin is very effective. All in contact animals should also be treated. Environmental acaracidal treatment is imperative- including grooming equipment, tack, stalls and pasture.
C. Chorioptic mange  
(Also named “Leg Mange”, “Tail Mange”, “Foot Mange”)  
Chorioptic mange is common in draft horses.  

i) Etiology: *Choriotes bovis*. Mites are host specific and do not affect man. They have a 2-3 week life cycle and can live off the host for a few days.  

ii) Clinical signs: erythema, alopecia, excoriations, crusts; moderate – severe pruritus. Subclinical carriers of the mite occur (possibly as high as 40-60% of an infested herd may be subclinical). Distribution: fetlock, pastern, perineum, back of the udder, and rear legs. Rarely, severe coronitis; feathers of draft horses may be site of asymptomatic carriage. The mite may extend proximally from rear limbs to involve the tail and perineum. Seasonality: most evident in the winter in stabled animals. In summer months, mites persist in the area above the hooves and can be found on skin scrapings in the absence of signs.  

iii) Diagnosis: Mites are easily found on superficial skin scrapings (mites are usually fast-moving).  

iv) Treatment of this superficial mite is challenging and treatment failures and relapses are common. Importantly, all animals in contact need to be treated concurrently. The treatment should be minimally extended to cover the life cycle (3 weeks). Also, since the mites can survive off the host for more than 2 months, it is wise to extend the treatment to cover this period of time. Moxidectin has been proposed as a suitable treatment but in one study moxidectin in combination with environmental insecticide treatment was found to be ineffective in the treatment of *C. bovis* in feathered horses. Failures are also seen with ivermectin (0.3 mcg/kg every 2 weeks for 3 times), most likely due to the superficial nature of the mites and their feeding habits. Lime sulfur has been shown to be effective to treat *Choriotes* when applied as a 5% solution weekly for 4 times. Shampoo with an antibacterial product that will help remove the crusts (e.g., benzoyl peroxide) is recommended before the dip. Lime sulfur dip will stain the hair and skin yellow and has an intense unpleasant sulfur smell. The dip should not be rinsed off to ensure residual activity. Fipronil has also been reported to be effective although this is an extra label use for this insecticide. If feathers are present it is advisable to clip the legs to facilitate topical therapy and better visualize and clean the area. Although clipping of the feathers is very helpful when treating this disease topically, resistance is frequently encountered as owners are typically concerned about the amount of time required for the feathers to grow back. Horses should not be kept in in muddy and wet conditions to allow dry conditions for the skin to heal.  

D. Demodectic mange  
Demodex mites reside in the hair follicles of horses, as they do in other species. Clinical disease, however, is very rare in this species and is only diagnosed in severely immunosuppressed horses. Two species of mites have been described: *D. caballi* affects the eyelids and the muzzle and *D. equi* that manifests as folliculitis on the body. Clinical signs are the ones of folliculitis and include papules, pustules, and alopecia. If *Demodex* is detected on skin scrapings it is important to diagnose and address the underlying immune suppressive disease. Typically once the disease is addressed, demodicosis will resolve spontaneously.
E. Forage mites
Forage mites such as *Pediculoides ventricosus*, *Pyemotes tritici* and *Acarus farina*, have been reported to cause dermatological disease in horses. These mites are free-living and found on straw and grain. The affected areas are ones that are in direct contact with the mites such as the face or the lower legs. A pruritic papular dermatitis can be seen in the contact areas and, in sensitized individuals, urticarial reactions may develop upon re-exposure. The final diagnosis is made upon microscopic demonstration of the mites in the forage or on the skin. Once the contamination is eliminated the dermatitis will resolve spontaneously. Severely pruritic individuals may require a short course of glucocorticoids.

F. Poultry mite
*Dermanyssus gallinae* can cause dermatitis in horses. Mites live in bird nests and if such nests are above the horse’s stall, mites can infest the dorsum causing a pruritic papular dermatitis. Diagnosis comes from skin scraping and the demonstration of the mites. Mites are easily killed by fly sprays. Decontaminate the environment is important to avoid re-infestation.

4. Pediculosis – Lice
Lice are highly host specific, obligate parasites that spend their complete life cycle (20 to 40 days) on the host. Infestations are common, but unless they are heavy, they are not harmful to the animal. Young animals in poor condition and on a low level of nutrition are most likely to have heavy infestations. Lice are a greater problem during the winter when crowding is a problem, nutrition may be poor, and animals have a long coat which provides a good environment for louse. Under favorable environmental conditions, lice can live 2 to 3 weeks off the host, but less than 7 days is more typical. Biting lice feed on exfoliated epithelium and cutaneous debris. Sucking lice feed on blood and tissue fluid. Nits are 1 to 2 mm long and are attached to hairs by a clear adhesive secretion by female lice. The lice are spread by direct contact or contact with bedding or other inanimate objects against which an animal has rubbed to relieve the itching sensation. Some animals remain infected year around and serve as carriers for the disorder. Pruritus and self-trauma result in excoriations and alopecia; coat becomes dull and skin becomes scaly. The animals can become debilitated if heavily infested. Sucking lice can cause anemia.

i) Etiology and clinical signs
(a) Biting louse of the horse: *Damalinia equi* prefer the dorsolateral trunk.
(b) Sucking louse of the horse: *Hematopinus asini* prefer the mane, tail, and fetlocks.

ii) Diagnosis; History, clinical signs, identification of the parasite. Examine coat meticulously (with good light source) for presence of adults. Eggs (nits) are glued to the hairs. Check skin folds and within ears. Vigorously brush scale and lice into a pan to facilitate collection and identification. Lice are usually easily visible; a magnifying glass may help in recognizing them.

iii) Treatment. Clipping the animal before treatment will improve efficacy. Approved, water-based insecticide sprays with pyrethrins and permethrin are effective. The coat should be thoroughly moistened, with special attention to the ears. Most insecticides are not ovicidal; therefore, treatment should be repeated in 2 weeks. The housing and bedding should also be sprayed. Ivermectin is useful for the treatment of sucking lice,
because populations are decreased > 90% after one injection and has shown promise in controlling lice. Ivermectin has been shown to be almost 100% effective in treating biting lice in calves when used as a pour-on.

5. Ticks
Ticks can cause dermatological disease in a variety of ways. The most common one is by inducing a nodular reaction at the site of the bite. The inflammatory response is determined by the interaction between the tick and the immune response of the host. In first exposures the main reaction is a toxic one, which manifests as necrotic changes in the epidermis and dermis and subsequent inflammatory response to the tissue damage. In animals that have been already exposed and have developed sensitization, the inflammatory response to the bite is more severe and persistent. Pyogranulomatous reactions are common and are demonstrated clinically by the development of hard nodules at the site of the bite that can open and drain a purulent exudate. Pruritus is variable.

Different types of hypersensitivities may be developed against tick bites. Some individuals can build type I hypersensitivity and, when challenged, develop a generalized pruritic papular reaction. Some individuals develop generalized urticaria and even angioedema. In some cases the urticaria may persist for weeks after the tick bite. Ticks can also trigger type III hypersensitivity which leads to vasculitis type lesions. Vasculitis can present as punctuate ulcerated lesions that, in severe cases, can coalesce and lead to the formation of large necrotic areas. Body sites that are prone to vasculitis are the extremities (e.g., tip of the ears and tail) and the lower limbs. Generalized malaise may be present as well as fever and edema. Secondary infections are common and will need to be treated aggressively. By their ability to transmit various viral, rickettsial and bacterial disease ticks are additionally able to trigger vasculitis through the development of these infections.

In terms of classification, ticks are divided into soft ticks and hard ticks. An example of soft tick (Argasid) is *Otobius megnini* also called the “spinous ear tick”. This tick lays eggs in crevices and he can cause severe otitis in horses. Clinical signs include severe inflammation of the ear canal, head shaking, and ear rubbing. In severe cases head tilt and muscle spasm have been described. Diagnosis is made by demonstrating the tick. Treatment involves the physical removal of the ticks and cleaning of the exudate. If a secondary infection is present, it needs to be properly diagnosed and treated.

Hard ticks are Ixodids and examples are *Ixodes, Dermacentor* and *Amblyomma*. *Ixodes* can transmit Lyme disease, which is due to an infection caused by *Borrelia burgdorferi*. Such infection is common in horses and ponies from the New England and mid-Atlantic regions of the United States. Although horses appear to be less predisposed to the development of diseases than humans are, they can still develop clinical signs. Symptoms include shifting lameness, poor performance, personality changes, laminitis, anterior uveitis arthritis, fever, edema, and encephalitis. In humans, the early symptoms is a characteristic circular skin rash called erythema chronicum migrans. This skin lesion occurs at the site of the tick bite a few days up to several weeks after the tick bite. The area is erythematous and warm, but is generally painless. These circular macules of erythema show some clearing in the center developing the appearing of a bull’s-eye. In horses cutaneous lesions are possible but are typically missed due to the presence
of the coat. Lyme disease is diagnosed based on a combination of clinical signs and blood tests to detect antigen specific antibodies.

Selected References


PRURITIC HORSE CASE DISCUSSIONS

There are no written proceedings for this session — please use this space for your own notes.
GENETIC SKIN DISEASES IN THE HORSE

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DermFocus, University of Bern, Switzerland

Introduction
The molecular genetics of two equine genodermatoses has recently been solved, hoof wall separation syndrome and incontinentia pigmenti. A brief review of these findings will be given followed by a compilation of some other genodermatoses whose molecular genetics is not yet solved.

Genodermatoses with Known Genetic Cause

Hoof wall separation syndrome (HWSD) in the Connemara pony (OMIA 001897-9796)
Danika Bannasch and her collaborators recently published the molecular genetics of this trait (Finno et al. 2015). The hoof in horses has evolved from the nail of the third digit. It represents a specialized organ in perissodactyla (odd-toed ungulates), which has been optimized to bear large weights while simultaneously extending the length of the limb and stride.

In Connemara ponies a monogenic autosomal recessive disease has been termed hoof wall separation syndrome (HWSD). In affected ponies the dorsal hoof walls at all four hooves start to separate at the sole at an age of 3-5 months. The horn of the hoof walls is very brittle and splinters off easily. These changes in the hoof wall may lead to laminitis and secondary infections, which are very painful for the horse and frequently require euthanasia. The phenotype of affected Connemara ponies is restricted to the hooves. All other organs including skin, hair, and teeth appear normal.

A genome-wide association study localized the causative genetic variant to chromosome 8 and whole genome sequencing revealed that HWSD is caused by a frameshift variant in the SERPINB11 gene encoding the serpin peptidase inhibitor, clade B (ovalbumin), member 11. The causative variant can be designated as c.504_505insC or p.Thr169Hisfs*3.

The biological function of the SERPINB11 protein is largely unknown so far. Members of the large serpin family can act as serine protease inhibitors, but also as molecular chaperones. The genes encoding the SERPINB sub-family are clustered in two genomic locations and the horse has more SERPINB genes than humans. This suggests that SERPINB genes may have been involved in the evolution of the hoof. The precise function of SERPINB11 in humans still remains elusive, but the equine data show that it is indispensable for hoof wall integrity.

Genetic testing is available at the University of Davis and breeders should be encouraged to use this test in a way that no more affected animals are bred. This does not require the categorical exclusion of carriers or even affected animals from breeding. However, carriers should always be mated to homozygous clear animals, so that no homozygous mutant offspring will arise.
Incontinentia pigmenti (IP) (OMIA 001899-9796)
IP is a well known genodermatosis in humans. The phenotype is characterized by 4 consecutive stages: perinatal inflammatory vesicles, verrucous patches, a distinctive pattern of hyperpigmentation, and dermal scarring. The mode of inheritance is X-chromosomal semi-dominant. IP patients are heterozygous females with one normal and one mutant X-chromosome. As mammalian embryos randomly inactivate one copy of the X-chromosome during early development, the developing fetus is a functional mosaic of cells expressing either the wildtype or mutant allele of the X-chromosome. IP is caused by loss of function variants in the \textit{IKBKG} gene encoding the “inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma” or NEMO protein, which is an essential modulator of the NFκB pathway. This results in the death of certain epidermal cells expressing the mutant allele around the time of birth. In females, these cells will be eventually replaced by cells expressing the wildtype allele. This process takes many years and ultimately results in characteristic scars following the lines of Blaschko. Hemizygous mutant males die during fetal development, which may result in resorption or abortion.

In horse breeding, a rare coat color phenotype termed brindle has been occasionally observed and is highly sought after by some breeders. This coat color phenotype is genetically heterogeneous. Some brindled horses exhibit similar scarring as human IP patients. Towers et al. (2013) demonstrated X-chromosomal semi-dominant inheritance of this phenotype in a Quarter Horse x Warmblood crossbred horse family. Similar to the human situation, all brindled horses in this family were female and there was a history of frequent abortions in several affected mares. Whole genome sequencing subsequently revealed that the affected mares carried a heterozygous nonsense variant in the \textit{IKBKG} gene (c.184C>T; p.Arg62*).

It has to be re-emphasized that also other types of brindling exist in horses (see below). While IP is clearly a genodermatosis that should not be bred on purpose, it is not clear whether other forms of brindling in horses have any negative impacts on health or wellbeing. There were anecdotal reports of genetically chimeric horses, which might have arisen from the fusion of two early embryos. It is conceivable that such horses can exhibit a brindled coat color without any negative health consequences. However, in this case the brindled coat color will not be transmitted to the offspring. Unpublished data from the author’s lab suggest that there probably is at least one additional heritable form of brindling.

**Genodermatoses & Hair Morphology Traits with Unknown Genetic Etiology**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Inheritance</th>
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<tr>
<td>Brindling (other than IP)</td>
<td>Inheritance not fully clear</td>
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<tr>
<td>Curly coat (American Curly Horse)</td>
<td>Monogenic autosomal dominant</td>
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<td>Hairlessness in Akhal-Teke</td>
<td>Monogenic autosomal recessive</td>
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**Selected References**

ENDOCRINE DISEASES IN SMALL Mammals WITH EMPHASIS ON FERRETS

Nico J. Schoemaker

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Introduction

In dogs, hypothyroidism is considered one of the most common endocrine diseases that affect the skin, followed by hyperadrenocorticism and hyperestrogenism (most often associated with testicular and ovarian tumors). For all of these diseases, alopecia without the presence of pruritus is a typical clinical sign.

In small mammals such as rabbits, rodents and ferrets, hypothyroidism is rarely diagnosed. Hyperadrenocorticism, in contrast, is one of the most important diseases seen in ferrets, with an estimated incidence of 75% in surgically neutered animals. Similarly, reproductive related endocrine disease is common in both (intact) ferrets and rodents and may include ovarian cysts (in guinea pigs) and persistent estrus (in jills).

Clinical Presentation of Endocrine Disease

Aside from systemic signs seen in endocrine diseases (e.g. lethargy, change in appetite, change in behavior and weight gain), gradual hair loss without regrowth of new hairs, eventually leading to (symmetrical) alopecia, is the most prominent sign. An important differentiating sign with infectious causes of alopecia is the absence of pruritus. An exception to this rule is hyperadrenocorticism in ferrets, as this disease will result in severe pruritus that is non-responsive to any of the regular antipruritic treatment options. Atrophy of the skin, which is a characteristic sign of hyperadrenocorticism in dogs, is not commonly seen in small mammals with endocrine skin disease.

Hyperadrenocorticism

Hyperadrenocorticism due to adrenal gland hyperplasia and/or neoplasia is considered the most widely reported endocrine disease in small mammals and has been reported in ferrets, rabbits, guinea pigs, and hamsters. Pituitary tumors have, thus far, not been associated with this disease in small mammal species. The disease in ferrets will be described in detail below, followed by an overview of presenting signs, diagnosis and suggested treatment in rabbits and rodents.

Hyperadrenocorticism in Ferrets

Hyperadrenocorticism is highly prevalent in neutered pet ferrets from 3 years and older, affecting male (hob) and female (jill) ferrets equally. In contrast to dogs and cats, hyperadrenocorticism in ferrets is characterized by elevation of plasma levels of androgens including androstenedione, 17-hydroxyprogesterone and/or oestradiol concentrations.

The most common clinical signs include symmetrical alopecia, recurrence of sexual behavior after neutering, and pruritus. Actual skin involvement is usually absent, although excoriations may be seen due to scratching. Other clinical signs include vulvar swelling in neutered jills and difficulty urinating in hobs due to peri-prostatic or peri-urethral cysts.
Etiologic considerations – Different etiologies have been suggested for the high incidence of hyperadrenocorticism in ferrets. These include (early) neutering of ferrets, housing ferrets indoors, and genetic background. In particular, (early) neutering has been associated with hyperadrenocorticism, whereby a castration-related increase of gonadotropins occurs as a result of a loss of negative feedback from the gonads, which subsequently stimulate the adrenal cortex, eventually leading to the development of a unilateral or bilateral hyperplasia or neoplasia of the adrenal gland.

Diagnostic work-up – Hyperadrenocorticism can usually be diagnosed based on the typical clinical presentation combined with the exclusion of other differential diagnoses such as persistent estrus, remnant ovary or food intolerance. During abdominal palpation, a (tiny) firm mass, representing the (enlarged) adrenal gland, may be palpated craniomedial to the cranial pole of the kidneys.

Hormone analysis is commonly recommended in the diagnostic work-up of ferrets suspected of hyperadrenocorticism. However, plasma concentrations of androstenedione, oestradiol, and 17-hydroxyprogesterone do not allow for differentiation between intact female ferrets or those with a remnant ovary or hyperadrenocorticism. Measurement of these hormones is, therefore, not considered helpful in the differentiation between the differential diagnoses. Similarly, measurement of urinary corticoid-creatinine ratios in combination with a high dose dexamethasone suppression test is not useful for differentiation purposes. Hormone analysis may, however, be useful for monitoring the effect of treatment.

Of the various techniques that are available, abdominal ultrasonography is considered the most useful tool in diagnosing hyperadrenocorticism in ferrets, particularly if surgical intervention is considered. However, it should be emphasized that ultrasound only allows establishment of the size and morphology of the organs and does not provide any information on the functionality of the tumor. The ultrasonographic changes of an adrenal hyperplasia or neoplasia include increased thickness, rounded appearance, heterogeneous structure, increased echogenicity, and/or the presence of signs of mineralization.

Therapeutic intervention – Treatment of ferrets with hyperadrenocorticism may include surgical intervention and/or the use of long-acting GnRH analogues (i.e. leuprolide acetate and deslorelin). The choice of treatment is influenced by many factors. Criteria such as the age of the ferret, presence of concurrent disease (e.g., renal failure, lymphoma and/or cardiomyopathy), risk of surgery (which is higher when the right or both adrenal glands are involved), and/or financial limitations may lead an owner to decline surgery. When surgery is chosen, however, it is important to realize that gonadotropin release will persist. This may subsequently lead to hyperplasia and/or neoplasia of the remaining adrenal gland at a later time. The use of hormonal therapy (in particular the placement of a long-acting implant containing deslorelin) may therefore also be recommended when a surgical intervention has been performed. The extra costs for the medication, may, however, result in an owner opting for surgery alone.

Prognosis – The prognosis of ferrets with hyperadrenocorticism is generally considered good, with an average disease free period of 16.5 months and 13.6 months reported for medical versus surgical treatment, respectively. In one study, 1- and 2-year survival rates after surgery were
Metastases rarely occur, but life threatening urinary blockage may occur in male ferrets, thereby negatively affecting the chances for survival if the condition is not treated promptly.

**Hyperadrenocorticism in Rabbits and Rodents**

Hyperadrenocorticism has also been reported in rabbits and rodents, though less frequently than in ferrets. In guinea pigs, an adrenal tumor has been described in a 4-year-old female animal which was presented with symmetrical alopecia and polyuria/polydipsia. The diagnosis was based on clinical signs, ultrasound and an increased salivary cortisol concentration. Treatment options that have been described include adrenalectomy and the use of trilostane.

In hamsters, hyperadrenocorticism has been described in a group of Teddy hamsters which were presented with symmetrical alopecia, hyperpigmentation of the skin, polyuria/polydipsia and polyphagia. The diagnosis was based on elevated urinary corticoid creatinine ratios. Both metyrapone or adrenalectomy have been opted as potential successful treatment, whereas ketoconazole and o,p’-DDD were considered ineffective.

In rabbits, the classical form of hyperadrenocorticism (increased concentrations of corticosteroids) has not been described, but return of sexual behavior associated with increased levels of androgens has been seen in neutered male and female rabbits over 6 years of age. The diagnosis was confirmed by measuring plasma sex steroids and abdominal ultrasound, which revealed an enlarged adrenal gland. Treatment options described in rabbits included surgery or the use of depot-GnRH formulations (deslorelin implants or injections with leuprolide acetate).

**Hypothyroidism**

Hypothyroidism has thus far only been (scarcely) reported in middle aged to older ferrets (> 3 years). The predominant signs included lethargy and obesity, although loss of hair was also seen. Diagnosis was made based on the lack of response following a TSH stimulation test. Similar to dogs, treatment with levothyroxine proved to be successful.

**Persistent Estrus**

Persistent estrus is commonly seen in unneutered jills that have not mated. Since estrus is only induced following successful mating, these jills will remain in estrus for a duration of up to six months. The persisting high plasma concentrations of estradiol will result in typical clinical signs including a swollen vulva, symmetric alopecia, pale mucous membranes, petechial hemorrhaging and lethargy. The latter are the resultant of the estrogen induced bone marrow suppression. Diagnosis is usually based on the history in combination with the clinical signs and the finding of pancytopenia. Treatment consists of suppression of ovarian function either by placing a deslorelin containing implant or surgical intervention (ovariectomy or ovariohysterectomy). The latter may pose significant risks, especially in case of a severe pancytopenia. Most owners are aware that (chemical or surgical) neutering of their jills prior to the first estrus will prevent this condition.

**Mammary Gland Tumors**

Mammary gland tumors are common in (older) rats of both genders, although female rats seem slightly more affected. The development of these tumors has been linked to the presence of...
prolactinomas, but clear evidence of this association seems to be lacking at this stage. Mammary tumors may be found anywhere on the body with the exception of the head, legs, tail and most dorsal part of the back. A tentative diagnosis can usually be made based on the clinical presentation. Cytological or histopathological examination of a fine needle aspiration or excisional biopsy may be used to confirm the diagnosis.

Treatment will often include surgical removal of the tumor, although some empirical evidence suggests that administration of a deslorelin-containing implant may also be effective. Cabergoline has been suggested post-operatively to decrease the likelihood of development of new mammary gland tumors. Since the association between prolactinomas and the development of mammary gland tumors is lacking and the amount of drug which needs to be given is high, there does not seem to be a basis for using this drug post-operatively. Neutering rats at an early age has experimentally been shown to reduce the incidence of mammary tumors in rats.

Ovarian Cysts in Guinea Pigs
Hormone producing ovarian cysts are the most likely cause for endocrine alopecia in female guinea pigs. However, most ovarian cysts in guinea pigs do not produce hormones and are; therefore, not likely associated with endocrine related signs. Ovarian cysts may be seen at any age, but are more prevalent in guinea pigs older than 3 years. The most prominent clinical sign is symmetric alopecia, which can sometimes be accompanied by abdominal enlargement. The diagnosis is usually based on the typical clinical signs in combination with abdominal palpation and visualization of the cysts using ultrasound. Ultrasound may also be useful when attempting to aspirate the contents of the cysts, but in some cases blind aspiration may also be successful. Hormonal treatments (e.g. injections with either hCG or GnRH) have been suggested in cases of follicular cysts, but are only effective in a small percentage of cases. As a result, ovariectomy or ovariohysterectomy are considered to be the most effective treatment. However, due to the risks associated with surgery and anesthesia, owners may opt to refrain from treatment at all. Unpublished work has shown that the use of depot GnRH-agonists is contraindicated as these were actually found to induce ovarian cysts rather than prevent them.

Pseudopregnancy in Rabbits
It is debatable whether pseudopregnancy is a disease, as it is a presentation of normal behavior in preparation of the birth of (non-existent) kits. Nevertheless, the disease is discussed here as it is commonly seen in rabbits and results in alopecia due to the pulling of hairs by the doe under the influence of progesterone. The doe may furthermore display nesting behavior and have enlarged mammary glands, which will assist in making the clinical diagnosis. The condition will usually resolve in 16 days without treatment, but cabergoline may be considered to reduce the clinical signs. Ovariectomy is advised afterwards to prevent recurrence of the condition, while at the same time also helping to prevent the development of uterus adenocarcinomas which are highly prevalent in rabbits.

Selected References


MANAGING UNUSUAL SMALL MAMMAL SKIN DISEASES

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1. Introduction
Skin diseases often produce similar skin lesions despite widely varying aetiologies and varying animal species. Armed with a little understanding of basic principles of skin disease, some knowledge of the common diseases, and the ability to perform just a few diagnostic tests and trials accurately, managing routine and unusual dermatology cases in a range of animal species can be simplified.

This presentation will briefly cover the more common skin diseases in unusual pet mammal species, and reinforce a sound diagnostic approach to skin disease, which is the vital first step to managing any skin case more effectively. The approach to diagnosis, diagnostic tests, and principles for treatment apply equally to other unusual mammal species, including wildlife and zoo animals. Consideration of the most likely underlying diseases allows much more targeted treatment, even when faced with financial or practical limitations that may prevent confirmation of a diagnosis. It is also relevant whether considering short-term symptomatic relief of acute disease or more effective management of chronic or recurrent problems.

Gathering a thorough history is the first step in a sound diagnostic approach to dermatological disease. Signalment (breed, age, sex), husbandry (housing, bedding, temperature, humidity, ventilation, cleaning, population density, diet, feeding frequency), skin disease details (duration, initial lesions, apparent pruritus, treatment, response, previous disease), and general health details are required. Step two is clinical examination, with a full physical and detailed skin examination required. Primary lesions (e.g. papules, pustules) are more useful diagnostic clues, and higher yield for diagnostic testing, than secondary lesions (e.g. excoriations, scaling, crusting). The vital third step, prior to reaching for diagnostic tests, is to make a preliminary prioritised list of differential diagnoses. This will guide the most appropriate diagnostic tests or trials and their priority.

For clinical relevance, it is often helpful to categorise differentials based on their typical presenting signs. The major presenting signs creating diagnostic challenges in unusual small mammal skin disease are pruritus, and alopecia/scaling/crusting, which are the major categories covered in this presentation. However, don’t forget that “common diseases occur commonly,” and thus atypical presentations of common skin diseases occur reasonably frequently, so this categorisation is a guide to how diseases most typically present rather than a firm division.

2. Pruritic Skin Diseases
Pruritus may manifest in a variety of ways including rubbing, scratching, biting, licking, rolling and shaking. Mild to moderate pruritus may not be readily evident to owners if animals are infrequently observed, so animals may be presented for signs associated with self-trauma due to pruritus. The most common cause of pruritic skin diseases in unusual pet mammal species is...
ectoparasites, followed by suboptimal environmental conditions. In contrast to dogs and cats, allergic causes are much less frequent. Pruritic dermatoses often present with minimal to prominent lesions. Secondary lesions frequently dominate, including alopecia, scaling, excoriation, and crusting; and lichenification and hyperpigmentation with chronicity. Primary lesions include papules and erythema. Skin biopsies are frequently unrewarding diagnostically, as pruritic skin conditions often produce similar histopathology.

**A. Parasitic Mites.** Mites are relatively common causes of pruritic skin disease in unusual mammals, and are one of the first important considerations faced with a pruritic patient. Knowledge of the more likely mites affecting different species is helpful (Table 1). All pruritic mites are superficial dwellers, living on hairs or in surface stratum corneum. Mites are contagious, sometimes zoonotic (Table 1), and life cycles are typically 2-3 weeks. Eggs will survive in the environment, off the hosts, for up to 21 days. Pruritus may vary from extreme to mild or absent within an infected group.

i) **Clinical signs.** Alopecia and scaling are often prominent, especially with more established infections, and self-trauma lesions (alopecia, excoriations) are usually reflective of the degree of pruritus.

ii) **Diagnosis.** Adhesive tape impressions, surface skin scrapings and ear swabs. Ear mites may also be visualised on otoscopic examination. Sarcoptid mites may be rare: treatment trials are required to exclude.

iii) **Treatment.** Treat all household pets and beyond the life cycle (minimum 4 weeks). Selamectin (Revolution®) appears effective for most mites (every 2 weeks for 3 treatments) and safe in all species. Fipronil (Frontline® spray) (full body spray) is effective except *not to be used in rabbits or hedgehogs*, and care is required to provide good ventilation and warmth for small species until dry. Subcutaneous ivermectin (every 2 weeks for 3 treatments) is reported as safe, but may not eradicate fur or surface dwelling mites, or sarcoptid mites in guinea pigs. Topical ivermectin is reported effective for ear mites. Ear canal cleaning is required for ear mites.

**B. Lice.** Louse infestations typically produce patchy disheveled fur and mild pruritus. Sucking lice may cause anaemia. Biting lice are less susceptible to systemic ivermectin. Topical selamectin (Revolution®) (every 2 weeks for 3 treatments) is reported as safe and effective. Treatment principles as for contagious mites: treat all animals of same species (lice are host species-specific), and treat beyond the life cycle (minimum 4 weeks).

**C. Other Infectious Pruritic Causes**

i) **Helminths** will occasional cause pruritic dermatitis. Pinworms (Rabbit: *Passalurus ambiguous*, Rat: *Syphacia muris*, Mouse: *Syphacia obvelata*) can cause perianal pruritus, self-trauma (and rectal prolapse in rabbits).

ii) Some common infectious diseases (e.g. bacterial pyoderma, dermatophytosis, and dermatophilosis) are usually associated with minimal pruritus. However, they may cause notable pruritus on occasions. It is important to remain alert for clues for such differentials (see Alopecic and Scaling Skin Diseases for more details).
Table 1. Ectoparasites infecting unusual small mammal pets

<table>
<thead>
<tr>
<th>Mites</th>
<th>Rabbit</th>
<th>Ferret</th>
<th>Guinea Pig</th>
<th>Rat</th>
<th>Mouse</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mites</strong></td>
<td></td>
<td></td>
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<tr>
<td>Sarcoptid (intense pruritus)</td>
<td>Rare</td>
<td>Rare</td>
<td>Common</td>
<td>Common</td>
<td>Hamster:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sarcoptes, Notoedres</td>
<td>S. scabiei* # (generalized; pododermatitis)</td>
<td>Trixacarus caviae* # (dorsal neck, face, thorax, generalized)</td>
<td>Notoedres muris # (nose, pinnae; rare tail, legs, perineum)</td>
<td>Notoedres notoedres # (ear), N. cati #</td>
<td></td>
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<tr>
<td>Ear Mites</td>
<td>Common</td>
<td>Common</td>
<td>Cheyletiella (surface scale)</td>
<td>Common</td>
<td>Chinchilla:</td>
<td>rare</td>
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<tr>
<td></td>
<td>Psoroptes cuniculi* # (ear canals, pinnae, rarely skin; rarely otitis media)</td>
<td>Otodectes cynotis* (ear canal, pinnae; rarely: feet perineum, tail)</td>
<td>C. parasitovorax* (dorsum)</td>
<td>If live with rabbits</td>
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<td></td>
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<tr>
<td>Fur Mites (hairs; mild pruritus)</td>
<td>Rare</td>
<td>Rare</td>
<td>Rare</td>
<td>Common</td>
<td>Gerbil:</td>
<td>Acarus farris</td>
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<tr>
<td></td>
<td>Listrophorus gibbus*</td>
<td>Lynxacarus mustelae</td>
<td>Chirodiscoides caviae</td>
<td>Radfordia ensifera (head, neck)</td>
<td>Myocoptes musculinus Myobia musculi # Radfordia affinis</td>
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<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td>Liponyssoides sanguineus ^ (also mice, rarely gerbils)</td>
<td>Psorergates muricola (pinnae)</td>
<td>Hamster, Gerbil: Demodicosis (see alopecic, scaling diseases)</td>
</tr>
<tr>
<td>Lice</td>
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<td>Sucking</td>
<td>Rare pets:</td>
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<tr>
<td></td>
<td>Haemodipsus ventricosus</td>
<td>Polyplax spinulosa</td>
<td>Polyplax serrata</td>
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<tr>
<td>Biting</td>
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<td>Common</td>
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<td></td>
<td>Glicicola porcelli; Gyropus ovalis</td>
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*Zoonotic potential  # Produce intense pruritus  + Vector for tularaemia ^ Vector for rickettsia

**D. Environment/Nutrition.** Suboptimal environmental and/or nutritional factors may create mild pruritus in any animal species due to compromise to normal skin health/skin barrier. Low humidity and inadequate diets may produce dry, scaly skin (ferrets, guinea pigs, hamsters). High humidity produces hair matting in chinchillas and gerbils (prefer natural dry environments). Moist, soiled, unhygienic bedding may
create an irritant dermatitis. Secondary bacterial infections can occur. Excessive burrowing may occur with inadequate bedding. Thus evaluation of any pruritic presentation should include evaluation of the pet’s environment, normal cleaning routines, and nutrition.

E. Behavioural. A number of normal or abnormal behaviours may mimic pruritic skin diseases.

i) Nesting behavior - hair plucking in rabbits

ii) Seasonal molting - rabbits

iii) Barbering from other cage-mates (more common in larger groups; produces alopecia +/- excoriations suggesting pruritus) - mice (common, dominant females), rats (less common), guinea pigs (lack of dietary fiber; dominant males), and rabbits (often subordinates), and gerbils, hamsters, chinchilla and rabbits (less common) - often produces complete well-demarcated regions of alopecia; self-barbering also suggested to occur with boredom

iv) Stress, crowding, and boredom - exacerbate excessive grooming and barbering in rabbits, guinea pigs, chinchilla

v) Repetitive behaviours (rubbing on cage bars or feeders, burrowing into abrasive bedding) – mice, gerbils (nasal dermatitis)

F. Allergies. Although very frequent causes of pruritus in dogs and cats, allergies are less frequent in unusual small mammal species.

i) Fleas readily occur on ferrets when housed with dogs and cats. Fleabite hypersensitivity is reported, with similar presentation to cats: barbered alopecia or papulocrusting dermatitis on rump, flanks, ventral abdomen, and caudomedial thighs. Treatment principles are the same as for dogs and cats, with a need to treat all household animals and the environment. Imidacloprid (Advocate®, a.k.a. Advantage®), selamectin (Revolution®) and fipronil (Frontline®) are considered safe for use in ferrets.

ii) Contact allergy/irritation to bedding materials is reported in hamsters to cedar or pine shavings (face and feet, pruritus, erythema and swelling), in rabbits to topical chlorhexidine, and in ferrets to shampoos. Similar presentations are likely in other small mammal species. Diagnosis requires exclusion of other causes, and resolution of signs on removal of offending agents.

iii) Atopy has been suggested to occur occasionally in ferrets. Exclusion of other potential diagnoses is very important before considering this differential.

G. Endocrinopathies. Adrenal gland disease is commonly linked to severe pruritus in ferrets. Adrenocortical hyperplasia or neoplasia produces excess sex hormones (oestrogen and/or androgen), and is reported to affect ~70% of ferrets in USA. Cortisol levels are usually within normal limits. Pruritus is reported in 40% of cases and may be severe and poorly responsive to symptomatic treatment (antihistamines, steroids). Bilaterally symmetrical alopecia, with dry dull hair coat begins on the caudodorsal trunk and gradually progresses cranioventrally. Details on diagnosis and treatment are well published. Excessive seasonal coat shedding is reported to cause similar alopecia during spring/summer breeding season, and will resolved completely
in autumn. Hyperoestrogenism in intact unmated female ferrets can also produce bilaterally symmetrical alopecia. Hyperadrenocorticism in hamsters, and cystic ovarian disease in guinea pigs also produce trunkal alopecia, although are not associated with pruritus.

3. Alopecic, Scaling, Crusting Skin Diseases

This group of diseases presents with prominent alopecia, scaling, and/or crusting, and typically without prominent pruritus. The major causes are infections (fungal and bacterial in particular), with occasional occurrence of sterile inflammatory or neoplastic dermatoses. General health is frequently reflected in the skin, thus systemic illness, nutritional deficiencies, and suboptimal environmental conditions may also produce similar lesions. If pruritus is present, it will often begin after lesions have developed. Fairly simple skin surface diagnostics will often identify infectious differentials. Skin biopsies may be helpful to clarify uncertain infections, and are essential for diagnosis of sterile inflammatory and neoplastic diseases.

A. Dermatophytosis. Dermatophytosis is one of the most common infectious causes of obvious skin lesions in unusual mammal species. It is common in guinea pigs and rabbits, and less frequent in rats, mice, hamsters, gerbils and ferrets. *Trichophyton mentagrophytes* is the most common causal species, followed by *Microsporum canis*, and *M. gypseum*, with a variety of other Microsporum and Trichophyton infections described; all with zoonotic potential to pet owners, especially children. *Trichophyton erinacei* infection is common in hedgehogs, and is often subclinical but with significant zoonotic risk.

i) Clinical signs. Patchy to complete alopecia, erythema and yellow crusting are often prominent. Pruritus ranges from absent, to occasionally severe. Infections are most typical on the face (periocular, nose, pinnae) but may be more generalized on dorsum and limbs/feet. Asymptomatic carriers are reported, with shedding/lesions during periods of stress.

ii) Diagnosis. The trichogram is well-described; adhesive tape impressions may be more sensitive and simpler to perform; Wood’s lamp will only detect some Microsporum species, and cautious interpretation of positive fluorescence is important; fungal culture is required for dermatophyte identification (false negatives, and occasionally false positives occur); biopsies can clarify, but fungal elements may be lacking in resolving areas.

iii) Treatment. Infections may be self-limiting in young animals, but risks of contagion and zoonosis often dictate treatment. All in-contact animals should be considered affected unless fungal culture suggests otherwise. Treatment recommendations are all anecdotal. Topical treatments (e.g. povidone iodine; miconazole or terbinafine creams; lime sulfur dips) may be effective for localized lesions if adequately and consistently applied. Antifungal shampoos (e.g. miconazole/chlorhexidine) may be adjunctive. Therapy recommended for more severe infections includes enilconazole rinse (twice weekly: chinchilla, ferret, gerbil, guinea pig, hamster, mouse, hedgehog, rabbit, rat), systemic griseofulvin (teratogenic: rabbit, ferret, chinchilla, guinea pig, hamster, hedgehog), and systemic itraconazole, fluconazole or terbinafine (rabbits, guinea pigs).
B. Bacterial Infections. Superficial bacterial skin infections in unusual pet mammals generally reflect suboptimal husbandry. A variety of bacteria may be involved, including *Staphylococcus aureus* (normal skin flora), *Pseudomonas aeruginosa* (common environmental moisture-associated contaminant), *Fusobacterium necrophorum* (common faecal organism in rabbits). Deep infections frequently present as abscesses or cellulitis. There are a variety of common presentations/causes, including:

i) Skin trauma
   a. Bite wounds - ferret, mouse, rat, guinea pig, gerbil, hamster, chinchilla
   b. Cage/substrate trauma - gerbil, hamster
   c. Pruritus/scratching - guinea pig

ii) Skin fold/moist dermatitis
   a. Rabbit - facial/dewlap, perineal areas from ocular or dental disease, obesity, unhygienic conditions, urine scalding; *Pseudomonas* spp. is often involved. Maggot infestations may complicate.
   b. Guinea pig, chinchilla - facial/chin from excessive salivation/dental disease

iii) Pododermatitis - common in guinea pigs (palmar/plantar feet), and less frequent in rabbits (plantar metatarsal surfaces); linked to suboptimal housing/substrates (wire-bottom cages, unhygienic conditions), obesity, and also vitamin C deficiency in guinea pigs. Lesions begin with regions of erythema and alopecia, and progress to erosions and ulceration. *Staphylococcal* spp. often involved in secondary infections. Scaly (keratotic) growths may occur.

Infections can spread to deeper tissues (abscesses, osteomyelitis, synovitis/tendonitis), and radiographs may be required to elucidate the extent of infection. Correction of underlying husbandry deficiencies, good wound care, debridement, and systemic antibiotics for deeper infections are all important for resolution. Topical silver sulfadiazine may be helpful. Obese animals should receive a high-fiber low-fat diet and exercise encouragement.

C. Demodicosis. Is very rare except in hamsters, but is also recorded in the ferret, guinea pig, and gerbil. It typically affects older, immunosuppressed animals. Non-pruritic alopecia, scaling and crusting occur. Mites are readily apparent on deep skin scrapings, although are can be found in normal hamsters.

D. Viral/Spirochete Infections
   i) Ferrets - distemper virus: erythemic pruritic crusting chin, ventral, mucocutaneous areas, progressing to generalized dermatitis; swelling/scaling of footpads are very characteristic. Mortality approaches 100%.
   ii) Rabbits
       a. Syphilis (*Treponema cuniculi*) is frequent in wild and domestic rabbits; infection is often subclinical; stress/overcrowding/poor sanitation can produce clinical disease: blisters, erythema, swelling and crusts occur on genitalia, perianal, nose and lips
       b. Myxomatosis - acute infections: periocular, facial, perineal oedema/swelling precedes death in most cases

E. Neoplastic Diseases: Epitheliotropic Lymphoma. Epitheliotropic lymphoma is reported rarely in older rabbits, ferrets, guinea pigs and hamsters. Presentation is variable, and may
mimic many other dermatoses, with alopecia and scaling often prominent. Nasal and footpad depigmentation may occur. Pruritus is variable. Diagnosis is reliant on skin biopsies.

F. Hair Cycling Disorders: Telogen Defluxion. Synchronization of hair follicles in telogen (resting) phase occurs 2-3 months after a stressful event (illness, surgery) or with pregnancy/lactation. Sudden shedding of hairs results, producing prominent areas of complete alopecia in the absence of pruritus (often lumbosacral area, flanks). Common in rabbits, and occurs in guinea pigs (pregnancy) and ferrets (pregnancy; generalized coat thinning rather than regions of alopecia). Exclusion of other common differentials is important (e.g. dermatophytosis). Resolution will occur within 2-3 months of shedding.

G. Environmental/Husbandry/Dietary Causes of Hair Coat/Skin Changes
i) Ferret - dietary deficiencies: low fat diet is associated with dull scaly hair coats (require high protein/fat, low fiber diets), and biotin deficiency (from excessive rare eggs) with symmetric alopecia, scaling
ii) Rats, Mice - ringtail (avascular necrosis of the tail) in young rodents is associated with low humidity/heating in winter. Dehydration is implicated.
iii) Chinchilla - fur slip occurs with rough handling; can rapidly shed patches of fur
iv) Gerbil - tail slip (skin degloving) occurs with rough holding; do not handle by tail

H. Sterile Inflammatory Diseases: Sebaceous Adenitis. Inflammatory destruction of sebaceous glands is reported in rabbits, presenting with non-pruritic scaly dermatitis that typically begins around the face and neck. Biopsy is required for diagnosis. One case was associated with thymoma detected at necropsy.

Selected References
Introduction
Pododermatitis, also referred to as bumblefoot (in birds) or sore hocks (in small mammals), is a condition characterized by the presence of infection, inflammation and/or degenerative changes of the ventral surface of the feet. The condition is commonly seen in both small mammals and birds. Without appropriate therapy, the disease tends to become progressive, not only involving the skin and connective tissues of the foot, but also the deeper tissues (tendons, joints and skeletal structures), thereby resulting in severe decrease or loss of limb function.

Aetiology and pathogenesis
In both birds and small mammals, pododermatitis has been linked to continuous or uneven pressure applied to the plantar surface of the feet. This pressure may subsequently result in disruption of the epithelial surfaces and ischemia and pressure necrosis of the soft tissue structures overlying the bony structures of the feet. As a sequela to the ischemia, and necrosis, inflammatory mediators are released, which, together with the oxidative damage caused during the intermittent reperfusion of the area, result in further tissue damage and vascular thrombosis. At this stage, chronic inflammation, hyperkeratosis, erosions and ulcerations may be seen. Invasion of bacterial organisms, in particular *Staphylococcus aureus* (all species), *Escherichia coli* (raptors, parrots) and *Pasteurella multocida* (rabbits), may subsequently result in secondary bacterial infections of the skin and underlying tissues. In severe cases, infection may spread to the underlying bones, tendons and joints, resulting in osteomyelitis, tenosynovitis and arthritis, thereby complicating the disease process and reducing the chances of the limb function returning to normal, especially if permanent damage to the tendons (displacement, rupture) is present. Occasionally, ulcerative lesions may also disrupt the integrity of the blood vessels of the foot, thereby resulting in significant haemorrhage and anaemia. In advanced cases, pododermatitis may lead to systemic spread of the infection with subsequent endocarditis or polyarthritis.

Predisposing factors
Many factors may play a role in the development of pododermatitis. In general, any management-related, medical or behavioural condition that results in an increased pressure of the weight bearing surfaces, alterations in the weight distribution or stance of the animal and/or increased friction and irritation of the skin may predispose an animal to developing pododermatitis (Table 1). Management-related factors that have been found to increase the risk for pododermatitis include lack of exercise, confinement to a small space, improper substrate or perches, poor or unsanitary housing and malnutrition. In addition, excess body weight, trauma and other physical or behavioural conditions that lead to an altered stance, activity and/or weight bearing (spondylosis, arthritis, neurologic disease, nervousness, conformational deformities) may increase the risk of the animal developing pododermatitis.
Although pododermatitis may develop in any bird or small mammal, certain species or breeds have been found to be more susceptible. For example, large and giant rabbit breeds (weighing >5 kg), raptors (e.g., falcons, eagles, osprey, red-tailed hawks), penguins, larger ducks and swans, and heavy-bodied psittacine birds (e.g., Amazon parrots, budgerigars, cockatiels) appear to be predisposed. Moreover, pododermatitis appears to occur particularly in rabbit breeds that have fine, sparse hair on the metatarsus (e.g., the Rex), thereby offering little protection against local injury, ischemia and pressure necrosis. Similarly, guinea pigs and rats are susceptible to sustaining injury to the plantar and palmar surfaces of their feet because of their relatively thin skin (similar to rabbits) and lack of hair on their feet and metatarsi. A recent study concerning risk factors of pododermatitis in rabbits from the United Kingdom revealed age and gender as predisposing factors, whereby they found pododermatitis to be more common in female, older rabbits. Moreover, neutering appeared to predispose the animals to pododermatitis. However, it cannot be excluded that this effect is the result from bias resulting from these animals also being more likely to be overweight. In other small mammals and birds, no age or sex predilection has been identified.

Table 1: Predisposing risk factors for pododermatitis in birds and small mammals

- Obesity or, in rabbits, emaciation
- Pregnancy (small mammals)
- Inadequate nutrition (hypovitaminosis A [psittacine birds], biotin deficiency [turkeys], hypovitaminosis C [guinea pigs])
- Lack of exercise
- Improper perches or substrate (inappropriately sized perches; abrasive surfaces such as carpets, sandpaper; hard floors such as tiles or cement; wire bottom cages)
- Moist and unhygienic conditions, leading to urinary and/or faecal soiling of the feet
- Poor husbandry (small enclosures, overcrowding)
- Unilateral lameness or pain, causing increased weight bearing to the contralateral limb (arthritis, fractures)
- Conditions that lead to reduced mobility, paresis, paralysis, or altered gait (spondylosis, neurologic disease, limb deformities)
- Trauma and abrasions to the feet (overgrown talons [raptors], puncture/bite wounds, trap injury, thermal and electrical burns/wounds, frostbite)
- Infections (*Staphylococcus aureus*, *Pasteurella multocida* [rabbits]; poxvirus [raptors, poultry]; *Knemodikoptes* [budgerigars]) – may both be the primary cause as well as secondary to erosion/ulceration of the plantar surface, thereby complicating disease
- Loss of protective hair covering (iatrogenic clipping of hair) in rabbits
- Behavioural: nervousness, excessive thumping (rabbits); bating of jesses (raptors)

Clinical presentation and staging
Pododermatitis encompasses a range of clinical presentations varying from mild erythema, swelling and/or superficial ulcerations to deep ulcerations with concurrent osteomyelitis, arthritis and/or tenosynovitis with loss of limb function. In birds, the plantar metatarsal pad or plantar digital pads are most commonly affected. In rabbits and rodents, the lesions are usually located on the caudal aspect of the tarsus and metatarsus and, less frequently, the metacarpus and phalangeal regions of the front limbs. In the initial stages, lesions in small mammals may be limited to focal hair loss (in rabbits), erythema, scaling and thinning of the skin of the plantar surface over the bony prominence of the metatarsus. In addition, erosion, dry crusty scabs or callous-like swellings, combined with mild to moderate inflammation may be noted in the earlier
stages of the disease. In more advanced cases, inflammation may be more severe and also involve the deeper structures, with obvious ulceration and tissue necrosis. Secondary infections may result in suppurative dermatitis, abscessation, tenosynovitis, osteomyelitis and/or arthritis. At this stage, loss of pedal function may also be observed (due to rupture or displacement of a tendon).

Dependent on the severity of the pododermatitis, mild to non-weight-bearing lameness of the affected limb may be seen. If pododermatitis is present bilaterally, animals may also show reluctance to move.

In both raptors and rabbits, classification schemes have been designed which help to grade pododermatitis depending on the extent, severity, and prognosis (Table 2 and 3).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Early lesion of the plantar surface involving integument only, with no secondary infection of deeper tissues. A loss of papilla, erythema and/or hyperkeratosis may be seen. Prognosis is excellent.</td>
<td>Excellent</td>
</tr>
<tr>
<td>II</td>
<td>Infection of the subcutaneous tissues with no gross swelling of affected feet. Localized signs of inflammation/infection and/or ischemic necrosis. Prognosis is good.</td>
<td>Good</td>
</tr>
<tr>
<td>III</td>
<td>Infected, swollen, painful feet without apparent damage to deep vital structures. Serous or caseous fluid draining from fibrotic lesion. Prognosis is good to guarded.</td>
<td>Good to guarded</td>
</tr>
<tr>
<td>IV</td>
<td>Infection of deep vital structures such as tendons and bones, though pedal function is retained. Patients may have concurrent tenosynovitis, arthritis, and/or osteomyelitis. Prognosis is guarded to poor.</td>
<td>Guarded to poor</td>
</tr>
<tr>
<td>V</td>
<td>End-stage disease with osteomyelitis, crippling deformity, and loss of pedal function. Prognosis is grave and euthanasia is often performed.</td>
<td>Grave and euthanasia is often performed.</td>
</tr>
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<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
<th>Prognosis</th>
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<tbody>
<tr>
<td>0</td>
<td>No lesions.</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>A small, circular area on the plantar aspect of the metatarsal bone-calcaneus (mono or bilateral lesions), with minimal alopecia, minimal epidermal hyperaemia and/or hyperkeratosis of the skin, but with no evidence of infection or bleeding of underlying tissues.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Circumscribed area of varying size localised at the caudal plantar aspect of the metatarsal-calcaneal area or extending linearly along the plantar aspect of the cranial metatarsal area with alopecia, erythema and scaling of surrounding tissues.</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Area of varying size focally ulcerated and with varying degree of keratinisation abnormalities. Infection of subcutaneous tissue present.</td>
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<tr>
<td>4</td>
<td>Full-thickness skin loss with swelling and necrotic debris may be present with infection of underlying tissues. Purulent exudates may be adherent to the lesions.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Severe infections with involvement of deep structures including bones and tendons with tenosynovitis, osteomyelitis and arthritis.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>End-stage disease with loss of pedal function.</td>
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</table>

**Diagnostic approach**

A definite diagnosis of pododermatitis can usually be made following the history and physical examination. Moreover, the history may reveal information on the potential risk factors such as nutrition, housing, bedding, and exercise. Similarly, a full and systematic physical examination is needed to discover concurrent diseases or predisposing factors for the pododermatitis. For this
purpose, the patient should initially be observed in its own cage or enclosure, whereby any changes in gait or abnormalities in resting or perching posture are recorded. A body weight and body condition score should also be obtained. Upon physical examination, special attention should be paid to the plantar (and palmar) surfaces of the feet to evaluate the presence of alopecia (in rabbits), erythema, erosions, ulcerations, necrosis, swelling and/or exudate. In addition, an orthopaedic and/or neurologic examination may be performed to assess the limb function and presence of (other conditions resulting in) pain and reluctance to move.

Diagnostic work-up may further include a complete blood cell count, serum chemistry panel, and urinalysis (if applicable) to identify concurrent diseases or predisposing factors. Moreover, impression smears and a culture and sensitivity of the affected area may help to determine involvement of bacteria and guide the choice of antibiotic therapy. Preferably, cultures are performed from swabs taken from deep within the lesion or pieces of infected tissue. If the lesion has extended into the deeper layers, imaging is recommended to identify involvement of the underlying bone or joints. Radiographs will usually suffice for this purpose (and may also help to assess presence of underlying limb and/or spinal disorders). However, computed tomography or magnetic resonance imaging may be considered to detect more subtle abnormalities of the bone and soft tissues.

**Management and prognosis**

Management of pododermatitis often requires a multimodal approach, which, dependent on the severity of the lesions, may include a combination of management, medical and/or surgical interventions. In any patient, treatment should be aimed at correction of the underlying causes and concurrent disease conditions. In addition, appropriate wound care and bandaging, topical medication, systemic antibiotics, analgesics and/or surgical intervention may be indicated.

**Correction of underlying causes** — Any underlying causes should be addressed appropriately. This generally involves adjustments to the animal’s housing, flooring, bedding and/or nutrition. For small mammals, cage floors should be adequately padded and/or bedded to reduce pressure on the feet (using foam rubber, thick towels, hay or fibre bedding). Bedding should be cleaned regularly to prevent risk of contamination and subsequent infection of the pedal lesions. In perching birds, perches of various sizes and types (wood, rope, rubber) should be provided. In addition, perches may be wrapped in safe, soft materials (cotton padding or bandaging tape) to provide extra protection. For raptors, flat perches covered with artificial turf (falcons) or round/elliptical perches made of hemp or sisal rope (eagles, hawks, owls) may be used. As for small mammals, surfaces should be kept clean and dry, and changed frequently. In waterfowl, access to swimming water (preferably a pool with gradual slopes) is important. Moreover, soft flooring or mats should be provided to reduce the pressure on the animals feet. Enclosures should be designed in such a way that movement, exercise and activity are stimulated. Enrichment (e.g. feeding stations, toys, foraging enrichment, tunnels, solid wheels) may also help to achieve this goal.

Aside from housing, correction of the diet is also important, especially in overweight animals. Moreover, dietary corrections may be aimed at resolving any potential nutritional deficiencies (e.g., hypovitaminosis C in guinea pigs, hypovitaminosis A in parrots) that may contribute to the problem.
**Wound care** – In general, pododermatitis lesions require open wound management and healing by secondary intent. It is important to keep the ventral foot surface clean and dry, especially if ulcerations are present. For small mammals, hair surrounding the lesions may be trimmed (though not down to the skin!) to prevent matting and contamination. In addition, the wound may be thoroughly cleaned using chlorhexidine or povidone iodine solutions (in the initial phases of treatment), or by soaking it in warm saline or lactated Ringer solution (in the later stages of treatment). Following wound irrigation, necrotic tissue may be removed.

**Bandaging** – Bandaging is usually necessary, except in mild cases. Their function is to prevent tissue desiccation and protect the wound against further pressure, trauma and contamination. Generally, bandages are composed of three layers: 1) a primary layer consisting of a dressing that is in direct contact with the lesion and provides protection as well as encourages drainage of exudate, delivery of medication and debridement of necrotic tissue; 2) a secondary layer that is composed of an absorptive material such as cast padding or cotton padding; and 3) a tertiary layer that keeps the bandage in place (Vetrap or similar product). In severe cases, additional protection may be applied in the form of interdigitating bandages, dental acrylic or polypropylene foam shoes, styrene plastic polymer foot casts, ball bandages and/or doughnut-shaped bandages. These bandages may all help to relieve pressure on prominent weight-bearing surfaces and redistribute pressure onto other surfaces. The type of bandage chosen usually depends on the stage of healing. For example, wet-to-dry bandages are often used in the initial drainage stage, whereas ball bandages, polypropylene shoes and interdigitating bandages may be used in the granulation stage, early and later stages of healing, respectively. Regardless of the type of dressing and bandage chosen, bandages should be changed regularly, starting with daily changes until the condition improves, following which the time between bandage changes can be prolonged to once every 2-3 days up to a week until the condition resolves. Preferably, both feet are bandaged to avoid development of pododermatitis of the other foot as a result of the shift in weight-bearing.

**Topical medication** – Topical medication may be used alone or in conjunction with a bandage. Examples of medications that can be used topically to treat pododermatitis include silver sulfadiazine, fusidic acid, mupirocin calcium, Manuka honey, antibiotic ointments (e.g. piperacillin, rifampicin, lincomycin), dimethyl sulfoxide (DMSO) and/or products containing proteolytic enzymes. Care must be taken when using topical antibiotics, because ingestion or absorption can result in toxicity in many species, whereas oil-based products may affect feather quality and impair thermoregulation in birds. Topical corticosteroids are generally not recommended because of their immunosuppressive effects and delayed wound healing. Footpad strengthening products may be applied during advanced stages of healing.

**Systemic antibiotics** – Systemic antibiotics may be indicated in cases of persistent or deep infections (grade 2 dermatitis or higher). Selection is preferably based on a culture and sensitivity, but a broad-spectrum antibiotic with action against Gram-positive and Gram-negative bacteria (e.g., trimethoprim-sulfamethoxazole) may be chosen. Antibiotic-impregnated beads (containing gentamycin, tobramycin or amikacin) also offer an effective method of antibiotic delivery to an infected ischemic area, especially in birds that underwent aggressive surgical debridement.
**Analgesia** – Analgesics comprise an important part of the treatment regimen. Generally, analgesia is provided through administration of nonsteroidal anti-inflammatory drugs (meloxicam, carprofen) and/or opioids (buprenorphine, butorphanol).

**Surgical intervention** – More advanced cases of pododermatitis (grade 3 or higher) often require aggressive surgical debridement to remove infected or necrotic tissue, promote drainage (especially in case of an abscess), stimulate formation of granulation tissue and improve circulation to the area. The wound may then be flushed with warm saline and antibiotic impregnated beads may be left behind in the wound. In addition, bandages may be placed to protect the wound and reduce pressure on the weight-bearing surface. Generally, wounds are left open to heal by secondary intent, but in some patients (primary) closure of the wound may be attempted after a granulation bed has developed. In severe cases (pododermatitis in conjunction with tendon displacement, severe osteomyelitis, septic arthritis and/or loss of limb function), limb amputation may be considered as a salvage procedure (Note: not advisable for wild birds!).

In addition to surgical intervention, low-level laser therapy or therapeutic laser has also been found to improve wound healing by promoting fibroblast development, collagen production, and epithelialization; enhancing leukocyte infiltration and macrophage activity; promoting angiogenesis and stimulating vasodilatation; and increasing lymphatic drainage of the region.

**Follow-up and prognosis**
Pododermatitis can be challenging and difficult to treat. Chronic pododermatitis will take several months to heal, depending on the severity and response to treatment. Prior to initiating therapy, owners should always be properly informed about the probable course and outcome. For mild to moderate cases of pododermatitis (grade 1-3) prognosis for recovery is generally considered good, especially if the underlying causes can be corrected. For grade 4-5 pododermatitis, the prognosis is considered guarded to poor, especially if osteomyelitis, septic arthritis and/or tenosynovitis are present. Euthanasia may be warranted in animals that suffer from severe, chronic pododermatitis, especially if more than one limb is affected, concurrent disease is present and/or the animal responds poorly to therapy and/or is not able to stand without severe pain.

Throughout the course of treatment, regular monitoring is advised whereby daily to weekly or biweekly rechecks are advised, dependent on the severity and stage of the disease process. Measuring the size of the lesion and documentation of the progress with the help of photographs are useful to evaluate the success or failure of the treatment plan. Aside from evaluating the lesion, assessment of the patient’s general condition, as well as the efficacy of the used protocol for pain relief, is considered essential.

**Prevention**
Proper husbandry, diet and client education are important tools to help prevent the development of pododermatitis. This includes the implementation of correct husbandry practices (using various perch types and materials, suitable flooring and soft, dry bedding, sufficient space), proper sanitation, offering the animal environmental enrichment and exercise (including flight in birds of prey) as well as a nutritiously balanced diet for the species in question, and preventing the animal from becoming overweight. In addition, periodic evaluation of the animal’s feet may
help to detect pedal lesions in an early stage, thereby increasing the chances of successful intervention.

Selected References

SCALING AND NODULAR DISEASES OF RABBITS AND RODENTS

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Scaling and crusting
Causes of scaling and crusting dermatoses include: Cheyletiella species mites, venereal spirochetosis (rabbit syphilis), hypovitaminosis C (guinea pig), sebaceous adenitis, cutaneous lymphoma, and thymoma.

Cheyletiella (non-burrowing mites): Cheyletiellosis in rabbits is a very common cause of mild to severe scaly dermatosis. It is zoonotic and contagious to other animal species such as dogs and cats. Diagnosis is by finding mites on skin scrapings or acetate tape preparations. In a recent article from South Korea, Cheyletiella parasitovorax and Leporacarus gibbus (another, less common, fur mite of rabbits), were found in 80 and 6, respectively, of 140 rabbits. Clinical signs of pruritus and scaling were observed in 17 of 80 and 76 of 80 infested rabbits, respectively. Both these mites can cause dermatitis in humans. Treatment is selamectin (see accompanying chart). Lime sulfur dips (1:32 dilution with water); 3 to 4 weekly dips is also effective, but messy and cumbersome in rabbits.

Other parasites that can cause scaling and/or crusting: Psoroptes cuniculi, Trixacarus caviae, Notoedres muris.

Treponema paraluisicuniculi (formerly, Treponema cuniculi) is the organism causing venereal spirochetosis (rabbit syphilis). Clinical signs include crusts, erythema, edema, papules, vesicles, ulcers and proliferative lesions localized to the face and perineum. In one study, lesions were found most frequently around the nose followed by the genitalia, lips, eyelids, and anus. Sneezing was observed in 33% of cases with nasal lesions. In cases of maternally acquired infection, lesions can be initially found mainly on the face. Lesions are painful but not pruritic. The disease may be associated with metritis, abortion and neonatal death. Rabbit syphilis is NOT zoonotic.

Diagnosis is by microscopic visualization of T. cuniculi from skin scrapes on dark field microscopy, or special silver stains to demonstrate the organisms on biopsy. Additionally, the serologic tests used to diagnose syphilis in humans can be used.

Treatment: Penicillin G at 40,000 to 80,000 IU/kg SC, weekly for 3 treatments. It is very important to monitor for signs of associated antibiotic enterotoxemia. Treat all in-contact rabbits. Chloramphenicol has been used successfully at a dosage of 55mg/kg q 12 h for 4 weeks. Another treatment is azithromycin 30 mg/kg/day given orally once or twice daily for 15 days; effectiveness in a large number of rabbits has not yet been reported, but this dose seems to be effective in experimental situations.

Sebaceous adenitis has been reported in domestic rabbits as a cause of alopecia and non-pruritic scaly dermatosis. Diagnosis is by biopsy. The author is unaware of a favorable response reported
to retinoids or glucocorticoids in the small number of rabbits treated. One report showed sebaceous adenitis and thymoma in the same rabbit. A similar presentation was seen in a rabbit with hepatopathitis. Histopathology showed a cell-poor interface dermatitis (lymphocytic infiltration and apoptotic cells in basal layer of epidermis), absence of sebaceous glands and lymphocytic mural folliculitis. A case report documents a rabbit with sebaceous adenitis that was successfully treated with a combination of cyclosporine and a supplement of medium-chain triglycerides. Another case report showed better success by adding topical application of a shampoo, spray and spot-on containing the ceramide precursor phytosphingosine.

Cutaneous lymphoma has been reported in hamsters, rabbits, mice, a guinea pig, and gerbils. It presents with severe alopecia, erythema and scaling. Prognosis is poor. There is one report of a hamster having both demodicosis (with D. aurati) and cutaneous lymphoma. An early report in rabbits noted a T-cell origin of the lymphocytes invading the epidermis, while a recent review of 25 cutaneous lymphomas in European pet rabbits classified the tumors as diffuse large B cell lymphomas, with 11 tumors exhibiting a T cell-rich B cell subtype.

Thymoma. A recent report describes 4 rabbits with confirmed mediastinal neoplasms — 2 thymomas, 1 thymic lymphoma, 1 unknown. All rabbits presented with multifocal alopecia, erythema, follicular casts and scaling. Histology of the skin showed orthokeratosis, lymphocytic exocytosis, lymphocytic mural and interface folliculitis, and absent sebaceous glands, similar to some previous reports of sebaceous adenitis in this species.

Nodular dermatoses
Causes of nodular dermatoses include: infectious/ulcerative pododermatitis, myxomatosis, mouse pox, trichofolliculoma (and occasionally other neoplasms such as fibromas and squamous cell carcinomas), as well as, congenital malformations. In hamsters, multiple trichofolliculomas have been associated with a polyoma virus.

Pododermatitis (“Sore Hocks”) has been reported in rabbits and guinea pigs, and noted in rats. It was the most common skin disease noted in a retrospective case study of rabbits and another in Guinea pigs.

Rabbits: Ulcerative pododermatitis is a chronic ulcerative granulomatous dermatitis of the metatarsal area seen in overweight inactive rabbits kept on wet bedding, grid floors, rough cages and/or unsanitary conditions. Hereditary factors are also thought to be involved and Rex rabbits are particularly affected as they lack protective guard hairs. The secondary infectious agent most commonly present is Staphylococcus aureus. Lesions are bilateral, in the plantar aspect of metatarsal area with a progression of lesions typified by erythema, hyperkeratosis, crusts, pus, necrosis, osteomyelitis and septicemia. Treatment is difficult and based on correction of predisposing conditions, surgical drainage, topical antimicrobials, surgical dressings, and systemic antibiotics (based on culture and sensitivity). Enrofloxacin (5-15 mg/kg subcutaneously once daily) may prove helpful in early cases. Antibiotic-impregnated methylmethacrylate (AIPMMA) beads have been reported as helpful. The earlier this disease is addressed, the better the chances of successful treatment. Pain management may be important; meloxicam (0.1-0.5 mg/kg PO q12-24 h) or tramadol (10mg/kg PO q24h) can be used. In a recent article investigating pododermatitis in pet rabbits, there was no statistical correlation between body
condition score and the presence of pododermatitis, but there was a statistically significant predilection in rabbits greater than 12 months of age, females, and neutered rabbits of either sex. Guinea pigs: Ulcerative pododermatitis is relatively common in guinea pigs. As in rabbits, *S. aureus* is generally isolated, although *Corynebacterium pyogenes* may also be found. Obesity, poor hygiene, hypervitaminosis C, and wire flooring are all predisposing factors. Lesions are bilateral, on the plantar aspects of the metacarpal and metatarsal areas with a progression of erythema, hyperkeratosis, pus, necrosis, osteomyelitis and septicemia. Treatment involves topical antiseptics (silver sulfadiazine or mupirocin may be helpful) and systemic antibiotic therapy (enrofloxacin as noted above) and bandaging, plus addressing the underlying cause. However, treatment is often unsuccessful, and systemic amyloidosis often occurs due to the chronic infection.

*Myxomatosis* is caused by a myxoma virus of the pox virus group, which is transmitted by various arthropod vectors, or through physical transport of the virus. New World rabbits are very resistant to this disease, but Old World rabbits are extremely susceptible (pet rabbits are Old World rabbits). There are various strains of this virus. Clinical signs in peracute and acute cases are edema of the head, ears, eyelids and genitalia and milky oculonasal discharge. Firm non-pruritic and erythematous nodules (myxomas) are usually associated with less virulent strains and develop at the site of infection. Lethargy, fever and anorexia can be present. Morbidity and mortality are high in pet rabbits, approaching 100%. The incubation period can range from 8 to 21 days. The diagnosis is based on clinical signs, typical microscopic lesions and virus isolation. Supportive treatment, vector control, and a vaccine (not commercially available in the USA) may be offered/discussed with the owner; the prognosis is grave.

Selected References
11. White SD, Guzman D, Paul-Murphy J et al. Skin diseases in pet guinea pigs (Cavia porcellus): A retrospective study of 293 cases seen at the University of California at Davis (1990-2015), submitted for publication.
Dosing of Selamectin
Utilization of REVOLUTION®*
(using the dog or cat pipette for the 5 lb and less-sized animal [60mg/ml])

<table>
<thead>
<tr>
<th>Weight of animal</th>
<th>Number of drops on 1 cotton-tipped applicator</th>
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<tbody>
<tr>
<td>&lt; 25 g</td>
<td>2 drops</td>
</tr>
<tr>
<td>25-50 g</td>
<td>4 drops</td>
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<tr>
<td>50-75 g</td>
<td>6 drops</td>
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</table>

Instructions:
- ✗ Put the exact number of drops directly from the pipette onto a cotton-tipped applicator
- ✗ Apply the applicator immediately to the skin of the animal between the shoulder blades while rubbing it in well.

<table>
<thead>
<tr>
<th>Weight of animal</th>
<th>Volume of Revolution® (measured as IUs using a 40 IU insulin syringe)</th>
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<tbody>
<tr>
<td>75-125 g</td>
<td>1 IU</td>
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<tr>
<td>125-250 g</td>
<td>2 IU</td>
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<td>250-375 g</td>
<td>3 IU</td>
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<td>375-500 g</td>
<td>4 IU</td>
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<td>500-625 g</td>
<td>5 IU</td>
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<td>625-750 g</td>
<td>6 IU</td>
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<td>750-875 g</td>
<td>7 IU</td>
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<td>875-1000 g</td>
<td>8 IU</td>
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<td>1000-1125 g</td>
<td>9 IU</td>
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<tr>
<td>1125-1250 g</td>
<td>10 IU (= 1 full pipette of 15 mg)</td>
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<td>1250-1325 g</td>
<td>1 pipette + 1 IU</td>
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<td>1325-1500 g</td>
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<td>1500-1625 g</td>
<td>1 pipette + 3 IU</td>
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<td>1625-1750 g</td>
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<td>1750-1825 g</td>
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<td>1825-2000 g</td>
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<td>2000-2125 g</td>
<td>1 pipette + 7 IU</td>
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<td>2125-2250 g</td>
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<td>2250-2375 g</td>
<td>1 pipette + 9 IU</td>
</tr>
<tr>
<td>2375-2500 g up to 4 kg</td>
<td>2 full pipettes of 15 mg</td>
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<tr>
<td>4-5 kg</td>
<td>1 pipette (for cats 2.5-7 kg) of 45 mg [60mg/ml]</td>
</tr>
<tr>
<td>+5 kg</td>
<td>1 pipette (for dogs of 5-10 kg) of 60 mg [120mg/ml]</td>
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Instructions:
- ✗ Puncture the pipette
- ✗ Take the volume indicated according to the animal’s weight using a 40 IU insulin syringe graduated in IUs; withdraw using the needle.
- ✗ Remove the needle and apply the product between the shoulder blades

Selamectin Table Source: after Bourdeau P, Houdre H. Unit of Dermatology/Parasitology/Mycology ONIRIS, Nantes-Atlantic College of Veterinary Medicine and Food Sciences, Nantes. France; World Association for the Advancement of Veterinary Parasitology 2003 Conference, New Orleans, LA, USA
*REVOLUTION®, Zoetis Inc, Florham Park, NJ, US
PLUMAGE DISORDERS IN BIRDS

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Introduction

Feathers are a distinct and unique feature that distinguishes birds from the other classes of vertebrates. They serve various functions including enabling of flight, insulation, waterproofing, camouflage and communication with conspecifics. Regular moultling, during which old feathers are shed and replaced by new ones, ensures that the plumage is in optimal condition. However, various disease conditions may affect the plumage of birds, resulting in various feather abnormalities such as stress marks, colour changes, broken feathers, malformations (feather dystrophy), feather loss, and/or improper moultling. For each of these, the most common aetiologies, diagnostic work-up, treatment and prevention is summarized in Table 1.

As can be noted in Table 1, the list of potential aetiologies for feather abnormalities is long and includes a variety of infectious, toxic, nutritional, neoplastic, immune-mediated, metabolic, endocrine, behavioural, traumatic and management-related conditions. To be able to effectively treat and resolve the problem, identifying the underlying cause for the feather abnormalities is vital. This necessitates a full-diagnostic work-up, starting with a full history and physical examination during which special attention is paid to the overall condition of the skin, feathers and feather follicles, as well as presence of signs indicative of an underlying systemic illness. Dependent on the findings, further diagnostic work-up may include collection of samples from the feathers and skin for culture and sensitivity testing, cytology and/or histopathology tests, testing for specific pathogens (Psittacine Beak and Feather Disease [PBFD]) or testing to identify underlying systemic illnesses or organ dysfunction (haematology and/or biochemistry, toxicology, faecal examination, diagnostic imaging, endoscopy). Following a correct diagnosis, treatment can subsequently be initiated to try and eliminate the underlying cause and resolve the feather abnormalities.
<table>
<thead>
<tr>
<th>Feather abnormality</th>
<th>Aetiology</th>
<th>Clinical presentation</th>
<th>Diagnosis</th>
<th>Treatment, prognosis and prevention</th>
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<tbody>
<tr>
<td>Stress marks (synonym: stress lines, stress bars)</td>
<td>Segmental dysplasia of the developing barb and barbules resulting from presence of (transient) illness, environmental stressors, malnutrition or iatrogenic administration of corticosteroids, fenbendazole during feather growth.</td>
<td>Presence of translucent lines in the vane of a feather, oriented perpendicular to the shaft. Particularly common in young, growing birds due to stress experienced during the weaning process.</td>
<td>Stress marks are usually readily identified upon spreading the wing and/or tail feathers and holding them against the light. Identifying the cause will generally be more difficult as the inciting event does not need to be present anymore.</td>
<td>Consequences usually only aesthetic, with spontaneous resolution occurring upon replacement of the old, affected feathers during the next moult. Larger numbers may indicate generalized disease, thereby warranting further work-up to identify and correct the underlying cause.</td>
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<td>Broken or abraded feathers</td>
<td>Often due to management errors (overcrowding, small cage size), iatrogenic trauma (during restraint, transport, crash landings) or self-inflicted damage. Other predisposing factors include malnutrition and stress, which result in weaker feathers that are prone to wear and tear.</td>
<td>Presence of feathers where part of the vane and/or shaft is damaged or missing. Caged birds are particularly prone. Allula and carpal coverts, primary and tail feathers are often involved. In case of extensive damage, difficulties with flight and balance may be noted. Severe haemorrhage may be seen if newly emerging pin or blood feathers are damaged.</td>
<td>A thorough history and dermatologic examination may help to identify any potential underlying causes (improper wing trim or weakening of the feather by abnormalities such as stress bars).</td>
<td>Consequences often merely aesthetic; damaged feathers will be shed during the next moult. More extensive damage to tail/flight feathers may require repair using a technique called ‘imping’. Bleeding may be controlled by manual pressure, application of coagulatory substances (flour) or pulling of damaged blood feathers. Eliminate predisposing factors to prevent future problems.</td>
</tr>
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<td>Poor feather quality</td>
<td>May arise from any condition that physically prevents a bird from preening (use of Elizabethan collars, beak malformations, obesity and/or orthopaedic problems). Other factors that may negatively affect feather condition are low humidity, exposure to aerosols, cigarette smoke or other toxins, malnutrition (common in birds fed an all-seed diet), feather or quill mites, and chronic, systemic illnesses.</td>
<td>Feathers with a ragged, unkempt appearance that may break easily. If the bird cannot preen, feathers may remain entrapped within their sheaths. Birds may furthermore present with delayed moulting, feather discolorations, and/or – in case of malnutrition – with thickening and scaling of the skin (face, feet, legs), or with secondary skin infections. In case of mites, birds may appear pruritic and restless.</td>
<td>A thorough history will help to identify errors in housing, care and/or nutrition, whereas the physical / dermatologic examination help to identify presence of parasites or underlying systemic illness. To diagnose presence of feather/quill mites, a microscopic examination of the pulp and/or vane of a damaged or developing feather may also be performed.</td>
<td>Following correction of the underlying cause, feather quality will usually improve; complete resolution may take one or more consecutive moults. An underlying nutritional component should be suspected in any bird fed an all-seed diet, and warrants treatment with multivitamin injections, oral vitamin/mineral supplements and conversion to a pelleted diet. Mite infestations are treated using oral, topical or parenteral avermectins (ivermectin, selamectin) and/or topical acaricides (permethrin, pyrethrin, fipronil).</td>
</tr>
<tr>
<td>Feather discolouration</td>
<td>Most commonly due to malnutrition. Differentials include liver disease, chronic lead toxicosis, hypothyroidism, neoplasia, drug administration (fenbendazole), early circovirus infection, genetic mutations and localized inflammation or trauma to a developing feather follicle.</td>
<td>Generalized or localized changes in feather colour. Changes may include depigmentation (riboflavin/choline deficiency in cockatiels), fading of the normal feather colour (low carotenoid intake) and true changes of feather colour (from green to yellow).</td>
<td>Diagnosis is usually based on a thorough history which reveals presence of underlying risk factors. Further diagnostic work-up may be needed to identify underlying metabolic, toxic or infectious disease.</td>
<td>In most cases, correction of the diet will result in a normally coloured plumage following a subsequent moult. If other causes are suspected, feathers should change back to their normal colour upon completion of a moult following the successful treatment of the underlying disease.</td>
</tr>
<tr>
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<tr>
<td>Feather cysts</td>
<td>Hereditary (genetic) in soft-feathered canaries (Gloucester, Norwich); incidental in other bird species as a result of an infection, trauma or other condition that interferes with normal feather growth.</td>
<td>Presence of an oval or elongated lump or mass filled with a yellow-whitish, caseous or firm material. If present on the wings, the cyst may interfere with flying. Secondary trauma or infection may occur.</td>
<td>Diagnosis is usually based on the typical appearance of the cyst, but cytology of a fine needle aspirate may be used for confirmation of the diagnosis.</td>
<td>Treatment will often involve curettage or surgical excision of the feather cyst. If multiple cysts are present, excision of the whole feather tract may be considered.</td>
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<tr>
<td>Folliculitis</td>
<td>Often resulting from (secondary) bacterial and/or fungal infections of the feather follicle. Common pathogens include <em>Staphylococcus</em>, <em>Aspergillus</em> and <em>Mallasezia</em> spp.</td>
<td>Presence of perifollicular swelling, erythema, pruritus and/or pain. In case of fungal infections, hyperkeratosis, crust formation and feather discoloration may also be noted.</td>
<td>Feather pulp cytology or histopathologic examination of a feather follicle biopsy may help to confirm the diagnosis; culture and sensitivity testing helps to identify whether and which pathogens are present.</td>
<td>Systemic and/or topical treatment with antibiotics (preferably based on the results of a culture and sensitivity test) or antimycotic drugs (itraconazol, miconazol or terbinafine). Often, treatment needs to be continued for 3 weeks or longer.</td>
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<tr>
<td>Polyfolliculitis</td>
<td>Exact aetiology is currently unknown, but viral infections (PBFD, polyomavirus) have been implicated as an underlying cause.</td>
<td>Multiple feathers appearing from the same follicle. Often, pruritus is present, resulting in feather plucking and/or automutilation. Commonly seen in lovebirds, budgerigars, and cockatiels.</td>
<td>Tentative diagnosis based on the typical signs; confirmation requires histopathology. PCR may reveal presence of a polyomavirus or circovirus infection.</td>
<td>Palliative treatment consisting of (manual or surgical) removal of the abnormal feathers, treatment of secondary infections and use of anti-inflammatory drugs to reduce inflammation and pain.</td>
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<tr>
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<tr>
<td>Feather dystrophy</td>
<td>May occur as a result of direct or indirect damage to the follicular collar or developing feathers. Viruses (circovirus [PBFD], polyomavirus) are often implicated, but in budgerigars, a hereditary component may also be present (‘feather duster’ or ‘chrysanthemum disease’).</td>
<td>Presence of abnormally shaped feathers. PBFD and polyoma primarily affect juvenile and young adult psittacines up to 3 years of age. PBFD usually becomes apparent after the first moult and gives rise to progressive and often bilaterally symmetrical feather, claw and beak deformities.</td>
<td>A presumptive diagnosis is usually made based on the distinct clinical features of the disease, but further diagnostic tests, including PCR, serologic testing or histopathology of a feather follicle biopsy, may be needed to obtain a definite diagnosis.</td>
<td>For chrysanthemum disease, no treatment is available; birds are therefore often euthanized in the nest. Similarly, no cure is available for PBFD or polyoma. Euthanasia is advised in advanced cases. Preventive measures include maintenance of closed flocks, quarantine, hygiene and disinfection, (repeated) testing and temporary cessation of breeding to reduce vertical and horizontal transmission.</td>
</tr>
<tr>
<td>Feather loss; lack of feather growth (inactive feather follicles)</td>
<td>In most cases, feather loss is self-inflicted. Differentials include: normal apertiae or normal moult; excessive or irregular moult induced by malnutrition or irregular photoperiod; trauma; genetic conditions; obesity; ectoparasites; PBFD or polyomavirus infections; mycoses; bacterial infections; neoplasia (xanthoma); systemic disease (hepatopathy, nephropathy); endocrine disease (hypothyroidism)</td>
<td>Localized or generalized loss of feathers resulting in focal, multifocal or generalized ‘alopecia’. Absence of newly forming feathers indicates inactive feather follicles, which may be the result of a PBFD infection or endocrinopathy (hypothyroidism). Occasionally, owners may note the bird (or its cagemate) to pull out the feathers itself.</td>
<td>Diagnostic work-up should include a thorough history and full physical/dermatologic examination to establish whether the feather loss is self-inflicted or results from lack of feather regrowth. Additional diagnostic work-up largely depends on differential diagnosis. Definite diagnosis of hypothyroidism warrants the use of a TSH-stimulation test.</td>
<td>Therapy mainly depends on the presumptive or definite diagnosis that is made. In any case, additional contributing factors such as suboptimal diet, housing, environment and management should be corrected as well. In case of hypothyroidism, treatment may be initiated with L-thyroxine.</td>
</tr>
</tbody>
</table>

* See the following notes in the CE Proceedings. YRA van Zeeland. Feather picking/feather destructive behaviours in psittacines.
Selected References
FEATHER PICKING / FEATHER DESTRUCTIVE BEHAVIOURS IN PSITTACINE BIRDS

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Introduction
Feather damaging behaviour (FDB; synonyms: feather destructive behaviour, feather plucking, feather picking, pterotillomania) is commonly seen in captive parrots, with an estimated prevalence of 10-15%. Although in many cases FDB predominantly has aesthetic consequences, medical issues may also arise due to alterations of the birds’ thermoregulatory abilities and metabolic demands, haemorrhage, and/or (secondary) infections. Moreover, FDB may indicate presence of behavioural issues indicative of a compromised welfare.

Clinical presentation
FDB comprises all activities with the beak that result in damage to the plumage. If the bird chews, frays and/or bites its feathers, feathers will generally look ragged with barbs removed, split shaft and/or v-shaped wedges cut out of the top of the feathers. Pulling of (contour and/or down) feathers, in contrast, results in patchy or generalized areas of feather loss, most commonly on the chest, ventral wing surface and inner thighs. Occasionally, damage will extend to the skin and/or soft tissues, thereby resulting in the behaviour being referred to as auto- or selfmutilation.

FDB is usually self-inflicted, resulting in varying degrees of feather damage across the body (except the head and neck). In rare instances in group-housed birds, FDB may be directed to cage mates or nestlings, whereby the head, face and neck are the main target.

Predisposing factors
FDB may occur in all species, but is particularly common in Grey parrots (*Psittacus erithacus*) and cockatoos (*Cacatua* spp.). Adolescent and adult female birds also appear more prone to develop FDB. Finally, personality traits, and in particular the individual’s coping strategy, have been suggested as a risk factor for developing FDB.

Aetiologic considerations
FDB is considered a complex problem, with various medical, socio-environmental, neurobiologic, and/or genetic factors contributing to its onset and maintenance.

Medical factors. As a general rule, any disease or condition causing pain, discomfort, irritation, and/or pruritus may result in development of FDB. This can include both primary feather and skin diseases (ectoparasites, dermatophytosis, skin neoplasia) and systemic diseases (endoparasitism, liver disease, endocrinopathies). In cases of systemic disease, feather damage may either be either diffuse and generalized (in case of Proventricular Dilatation Disease) or localized directly over the region of discomfort (in case of renal disease). In most cases,
however, evidence for a true causal relationship between the medical condition and FDB is lacking.

*Socio-environmental factors.* Various features of the environment are thought to play a role in the development of FDB. For example, a small cage or poor cage design may result in damage to the feathers, which are subsequently removed by the bird as part of normal preening behaviour. Moreover, FDB may occur as a result of nutritional deficiencies and dietary imbalances; exposure to airborne and topical toxins; poor wing trims and/or low humidity. Confinement and limited access to essential stimuli (social contact, foraging opportunities, locomotor activities) may also play a role as this limits the bird’s ability to engage in species-typical behaviours, thereby inducing stress, boredom, and/or frustration.

In addition, exposure to aversive stimuli (novelty, overcrowding) and/or sudden changes in the environment (lack of predictability) may result in anxiety and/or stress-induced FDB. In these situations, FDB may be regarded as a coping or tension-release mechanism to deal with negative affective states resulting from an inadequate living environment (maladaptive behaviour), whereby the behaviour can be exacerbated by the owner’s responses through the process of reinforcement.

*Neurobiologic factors.* Upon prolonged exposure to a suboptimal living environment, FDB may eventually develop into abnormal repetitive behaviour (ARB), which comprises stereotypies and impulse control disorders (malfunctional behaviour). In ARBs, an altered neurochemistry and neuroanatomy may lead to persistence of the behaviour, even in absence of the original stressors or environmental deficits. Aside from the current living environment, early living environment (in particular hand-rearing) also exerts a considerable effect on the bird’s behavioural development and poses a risk factor for FDB.

**Diagnostic approach**

The focus of the initial investigation is to establish whether the feather damage is inflicted by the bird itself (or its cage mate) or due to a medical or environmental condition that causes loss or damage to the bird’s plumage irrespective of its behaviour. It may, however, be challenging to determine whether the damage is self-inflicted as caregivers are usually not able or willing to observe the bird 24/7 and may experience difficulties when trying to distinguish FDB from normal preening. The physical examination may, however, confirm the potential self-inflicted nature of the feather damage and/or loss by the absence of feather abnormalities on the head and crest, which are inaccessible to the bird’s beak.

Next, the investigation should focus on identifying whether the condition a) primarily originates from a medical condition; b) results from husbandry, management and/or nutrition related issues; c) if it should be regarded as a primary behavioural problem (psychogenic FDB or pterotillomania); or d) a combination of two or more of the above. A thorough history and full physical examination (including a thorough dermatologic examination) are deemed essential for this purpose, as these help to identify presence of one or more factors that contribute to the problem. Dependent on the findings and remaining differential diagnoses, additional diagnostic work-up may include collection of diagnostic skin and feather samples (in case a primary skin or feather condition is suspected) or the collection of blood, faecal or urine samples, imaging and/or
endoscopy (in case a systemic disease is suspected as the underlying cause). Although allergic skin disease and hypersensitivity are highly suspected in birds, obtaining a definite diagnosis remains difficult as intradermal skin testing has been found unreliable. However, paired skin biopsies from affected and unaffected areas of the same patient may help to identify the presence of inflammation consistent with delayed-type hypersensitivity reaction, indicative of an allergy.

If obvious medical factors are lacking, a psychological or behavioural origin becomes likely. At this stage, it will be important to identify any potential underlying triggers (antecedents) and reinforcing factors (consequences) that could have contributed to the onset and maintenance of FDB. Applied behaviour analysis, which focuses on the environmental determinants of behaviour and the behavioural changes that occur as a result of learning, is considered helpful to generate hypotheses regarding what is going on and why, which are two key questions that need to be answered in order to design a successful and effective behaviour intervention plan.

**Therapeutic considerations**

Choices with regard to therapy for the individual feather plucking bird will largely depend on the findings obtained from the history, physical examination, and diagnostic tests. Initial treatment will generally be aimed at optimizing the birds diet, housing and living conditions, thereby addressing the environmental factors that may contribute to the development and maintenance of the behaviour. In addition, treatment may consist of a combination of interventions to treat underlying medical conditions, provision of enrichment, behaviour modification strategies, use of psychoactive drugs and/or devices that physically prevent the bird from damaging its feathers and/or skin.

*Interventions aimed at treating underlying medical conditions.* If medical conditions are encountered during the work-up of a feather damaging bird, these should always be appropriately addressed. The choice for a specific therapy will depend on the underlying disease. In case of an underlying parasitic, bacterial or mycotic disease, treatment may include the use of topical and/or systemic antibiotics, antifungals, and/or antiparasitic drugs. If allergies are suspected, antihistamines and/or corticosteroids may be considered in combination with dietary and/or environmental modifications that reduce the exposure to the suspected allergen(s). However, caution is advised when using corticosteroids in birds as these may have profound immunosuppressive effects in birds, thereby rendering them susceptible to secondary infections, in particular aspergillosis.

*Enrichment strategies.* Various types of enrichment may be provided in order to provide a more stimulating environment to the parrot and reduce FDB. Examples include social contact, auditory stimulation, climbing enrichment and perches, chewing toys, and foraging enrichment. Foraging enrichment in particular has been found effective to reduce FDB and may be provided by offering the bird complicated food items (e.g., corn on the cob, walnuts), providing food in larger chunks, mixing food with inedible items, scattering food and/or using multiple feeding stations, and providing food in home-made or commercial foraging devices (puzzle feeders).

*Behaviour modification therapy.* Aside from environmental enrichment, behaviour modification techniques comprise an important part of a treatment regimen for a feather damaging bird. Examples of behaviour modification techniques that may be employed are differential
reinforcement of alternative behaviour, systematic desensitization and counterconditioning. Preferred techniques include those using positive reinforcement, whereas punishment strategies are preferably avoided.

Physical restraint devices. In the past, Elizabethan collars and neck braces have commonly been used to prevent birds from damaging their feathers. Foul smelling substances have also been advocated for a similar purpose. However, these interventions merely help prevent the symptoms rather than help to eliminate the underlying cause. As a result, these are only recommended as a temporary measure to stop birds from automutilating themselves and/or break the cycle of habitual FDB. In case collars are needed, the use of tranquilizer drugs such as midazolam can be helpful. In addition the use of fabric “ponchos,” “jackets,” or “vests” may be considered as these are a more bird-friendly method to prevent a bird from harming itself.

Psychoactive drugs. Pharmacologic intervention may be considered in patients that do not respond to treatment with behaviour modification therapy and environmental changes. Depending on the underlying motivation, options for psychopharmaceutic drug treatment in birds include: a) anxiolytic drugs [diazepam]; b) antipsychotic drugs [haloperidol]; c) tricyclic antidepressants [clomipramine]; d) serotonergic reuptake inhibitors [paroxetine]; and e) opioid antagonists [naltrexone]. Aside from psychoactive drugs, hormone therapy (depot gonadotrophin-releasing hormone agonists) may also be considered in birds suspected of sexual or hormone-related FDB.

Prognosis and monitoring
Prognosis is often considered guarded as a result of the multifactorial origin of the disease, difficulties experienced with the timely and accurate diagnosis of the underlying causes, the chronicity of the problem which poses an increased risk for ritualization of the behaviour, and the overall lack of scientific evidence regarding the efficacy of the different treatment options. To evaluate the effects of a proposed treatment plan in the individual bird, adequate monitoring and follow-up are deemed essential. For this purpose, direct behavioural observations may be useful, but comprise a labour-intensive task of which the reliability is doubtful. Feather scoring systems, on the other hand, provide a more practical and reliable alternative, enabling changes in feather score to be monitored over time as long as sufficient time is present between two consecutive check-ups to allow the feathers to regrow.

Selected References


OF SCUTES AND SCALES: DERMATOLOGIC CONDITIONS IN REPTILES WITH EMPHASIS ON CHELONIANS

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Introduction
Of the exotic animal species, reptiles are next to fish, the least commonly seen species by veterinarians. In the past 20 years, however, owners of these species have realized that for appropriate care, veterinary consultations are mandatory as well. The amount of reptiles presented to veterinarians is; therefore, increasing.

The order of reptiles is extensive and consists of at least 10,000 species. At least 250 different chelonian species are recognized of which some are terrestrial, some are aquatic and some are semi-aquatic. The different circumstances under which they live effect the structure and function of their skin and shell. Consequently, this also effects the type of diseases seen in the different species.

Prior to discussing the dermatological conditions seen in chelonians, and other reptiles, the anatomy of the reptilian/chelonian skin is discussed, as this differs in some areas greatly from mammalian (and avian) skin. After discussing the anatomy, a list of potential aetiologies for dermatological conditions in reptiles/chelonians is provided which includes a variety of infectious, toxic/nutritional, neoplastic, and management-related conditions. For the latter, it is important to be familiar with the requirements of the species with regard to temperature, relative humidity and substrate on which the reptile/turtle should be kept. An overview is provided in table 1.

Skin Anatomy
The skin of reptiles is adjusted to the circumstances under which the different species live. The outer epidermis may be thick and scaled in terrestrial tortoises to resist the abrasive circumstances under which they live. In contrast, the skin in aquatic species is thinner and smoother. Skin glands are very scarcely present in reptiles. Shedding, also known as ecdysis, in chelonians is continuous and seemingly without a pattern.

The skull and shell of chelonians are ossifications of the dermis whereby the ribs are joined into the shell. The dorsal part of the shell is called the carapax, while the ventral shell is called the plastron. The shell is covered by epidermal, keratinized plates known as scutes. During growth new epidermal plates are added. In some aquatic chelonians, such as the soft-shelled turtle, the shell is less ossified, but rather has a leathery structure. Some turtles, such as the box turtle, have hinges in their plastron enabling them to close the entire shell, thereby making their soft structures inaccessible to predators (but also to veterinarians).
Examination and Diagnostic Tests
The history of the patient plays an important role in determining the cause of the dermatological condition of the patient. During a thorough history special attention should be paid to the management, including diet and husbandry (including substrate, lighting, heating, humidity and temperature) as these can both be primary as well as contributing factors in the development of dermatologic disease.

Following the history, a full physical examination (to detect underlying systemic disease) and dermatological examination should be performed. Common findings during the clinical examination of reptiles with dermatologic disease include abrasions, erosions, ulcerations, wounds, swellings, blisters, crusts, petechial haemorrhaging, discolorations and dysec dysis (abnormal shedding of skin).

Following the physical examination, samples may be collected for further evaluation. Commonly used diagnostic tests include cytology of fine needle aspirates, impression smears or skin scrapings, parasitological evaluation of acetate tape impressions or shed skin fragments (particularly helpful to diagnose mites), histopathologic examination of skin biopsies and skin cultures to detect bacterial or fungal organisms. Evaluation of radiographs, haematology and biochemistry are considered useful when suspecting underlying systemic disease (e.g. metabolic bone disease, renal disease).

Following a correct diagnosis, treatment can be initiated to treat the symptoms as well as try and eliminate the underlying cause, as well as secondary bacterial or fungal infections.
<table>
<thead>
<tr>
<th>Skin abnormality</th>
<th>Aetiology</th>
<th>Clinical presentation</th>
<th>Diagnosis</th>
<th>Treatment, prognosis and prevention</th>
<th>Other remarks</th>
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<tbody>
<tr>
<td>Abrasions (rostral)</td>
<td>Inadequate environment leading to rubbing, pushing and/or banging onto abrasive surfaces, or the wall of the vivarium. Also common in highly active species which are easily startled</td>
<td>Superficial to deep lesions to the tip of the nose</td>
<td>Clinical aspect Culture and sensitivity Radiographs to assess bony involvement</td>
<td>Clean and disinfect wounds with povidone iodine; Topical and/or systemic antibiotics to treat secondary infections; Apply plastic skin dressings (mild lesions) or epoxy resin (severe lesions); Modify the environment (e.g. visibility of glass, remove abrasive surfaces; provide hiding areas; separate aggressive cage mates).</td>
<td>If allowed to, progress wounds may affect deeper layers and involve bone which will result in a poorer prognosis</td>
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<td>Blisters</td>
<td>Moist bedding and/or a humid environment (particularly in desert species); thermal burns; secondary bacterial and/or fungal infections.</td>
<td>Vesicles’ or blisters, predominantly on the ventral side; may progress to abscesses and/or ulcers</td>
<td>History Inspection of vivarium Culture and sensitivity</td>
<td>Daily baths with povidone iodine; Systemic or topical antibiotics (based on culture); Address husbandry issues (e.g. optimize temperature, humidity)</td>
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<tr>
<td>Burn wounds ([thermal] burns)</td>
<td>Close/direct contact with unprotected heat and/or light sources (e.g. hot rocks, heat mats) Caustic substances Faulty electric equipment</td>
<td>Burn wounds, blisters and ulceration of the skin, varying from superficial to deep ulcers</td>
<td>History Inspection of vivarium Culture and sensitivity</td>
<td>Supportive care (e.g. parenteral fluids); topical and/or systemic antibiotics; pain relief; wound management (e.g. wet-to-dry bandage, surgical debridement). Prevent direct contact with heat source; May lead to scarring and dysecdysis; Secondary infections due to <em>Pseudomonas</em> are common</td>
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<td>Colour change</td>
<td>Trauma Injection site reaction Systemic disease Ectoparasites Hypothermia Thermal burns Stress</td>
<td>Discolouration of the skin; redness or darkening of the skin</td>
<td>History Inspection of vivarium Inspect skin for mites Haematology &amp; biochemistry</td>
<td>Treatment of the underlying cause</td>
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<td>Contact dermatitis</td>
<td>Substrates containing aromatic compounds, Residues of cage cleaning products (e.g. bleach/phenols)</td>
<td>Erythema, blister formation between scales, exudate oozing from inflamed (infected) skin; petechial haemorrhaging</td>
<td>History, Inspection of vivarium</td>
<td>Treatment similar to (thermal) burns; Removal of the underlying cause</td>
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<tr>
<td>Damage to the distal end of extremities including the tail</td>
<td>Avascular necrosis (dysecdysis, hypothermia), Septicaemia, Trauma (e.g. bite wound; incorrect handling leading to loss of tail = autotomy)</td>
<td>Necrotic tail tip with dry, often black discoloration; Autotomy in lizards</td>
<td>History, Clinical presentation, Haematology &amp; biochemistry</td>
<td>Treat the underlying cause. Stimulate peripheral circulation (isoxsuprine); Amputation cranial to the demarcation line of affected tissue. Use analgesia and antibiotics if secondarily infected</td>
<td>In lizards: no suturing of the amputation site as this will prevent regrowth of the tail</td>
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<td>Dysecdysis (abnormal shedding of scutes and scales)</td>
<td>Low temperature and humidity (high humidity in chelonians); stress; inadequate furnishing to rub against (in snakes); (scars due to previous) trauma, wounds; systemic disease (e.g. nephropathy); malnutrition; bacterial, fungal and/or parasitic skin disease</td>
<td>Abnormal skin sloughing; loss of scutes. Most commonly seen in snakes and lizards. Retained pieces of skin may encircle toes or tail and lead to avascular necrosis. Retained spectacles are typical in snakes.</td>
<td>History, Inspection of vivarium</td>
<td>Correction of the underlying cause and treatment of secondary infections (povidone iodine and antibiosis). To aid in shedding, bathe in warm water, or place in warm, moist environment. Careful manual removal of skin is possible, but when removing retained spectacles, care must be taken to prevent corneal lesions and loss of the eye.</td>
<td>Dull appearance of skin prior to shedding is normal. New skin more susceptible to secondary infections and sensitive to the use of topical antiparasiticides</td>
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<td>Epidermal and dermal separation</td>
<td>Hypervitaminosis A (parenteral injection in tortoises); hypovitaminosis C (in boids); renal disease; cachexia; hypoproteinaemia</td>
<td>Dry or flaky skin followed by severe skin loss and blisters (in tortoises). In renal disease: oedema, polyuria or anuria</td>
<td>History, Clinical signs</td>
<td>Cleaning and debridement of the wounds, followed by application of topical antibiotic and dressing. Fluid therapy and systemic antibiosis may be warranted in severe cases. Vitamin C suppletion (in snakes)</td>
<td>Caution with use of multivitamin injections containing vitamin A in tortoises</td>
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<tr>
<td>Hyperkeratosis, crusts, scales</td>
<td>Hypovitaminosis A; Fungal infection (e.g. Aspergillus, Fusarium, Chrysosporium, Monelia; Mucor, Nannizzopsis, Penicillium) Underlying causes: primary skin damage/infection; poor nutrition; poor housing (water quality / pH in aquatic spp.); stress</td>
<td>Crusts, white scaly skin lesions, dull roughened scales, hyperkeratosis with yellow/orange/brown discoloration, multifocal circular grey lesions (carapax, plastron), necrosis; lateral/ventral scales most commonly affected.</td>
<td>History Inspection of vivarium Clinical signs Culture and sensitivity</td>
<td>Remove necrotic tissue; Bathe in dilute povidone iodine Topical and/or systemic antifungal treatment (miconazole, nystatin, voriconazole, enilconazole, ketoconazole) Correct husbandry (water quality control in aquatic species) and diet</td>
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<td>Mites</td>
<td><em>Ophionyssus natricis</em> in snakes; <em>Ophionyssus acertinus</em>, Trombiculidae and Pterygosomatidae in lizards</td>
<td>Red or black mites are seen moving on the animal (in lizards predominantly on the head and around the eyes); Severe infestations may lead to skin rash, pruritus, anaemia, dysecdysis</td>
<td>Visualization of the mites on the animal or in the environment</td>
<td>Fipronil or topical/parenteral ivermectin; Also treat the environment (e.g. permethrin, pyrethroids and/or pyrethrins); Disinfect tank and furnishings, replace substrate</td>
<td>Note: Ivermectin is toxic for chelonians!</td>
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<td>Myiasis</td>
<td><em>Lucilia</em> spp lay eggs in wounds and moist areas. Poor hygiene, diarrhoea, faecal soiling and wounds will attract the flies.</td>
<td>Maggots infesting wounds, leading to extensive tissue damage at the site of infestation</td>
<td>Visualization of maggots</td>
<td>Remove the maggots, flush with dilute povidone iodine. Topical or systemic antibiotics may be needed. Improve hygiene.</td>
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<td>Overgrown beak and claws (in chelonians)</td>
<td>Suspected to be caused by soft food diets and decreased foraging times; Lack of abrasive substrate; Excess dietary protein</td>
<td>Overgrown keratinous mouthparts, especially maxilla; may lead to severe malocclusion and subluxation of the temporomandibular joint</td>
<td>History and Clinical signs Diagnostic imaging to help evaluate the temporomandibular joint</td>
<td>Beak and nail trim to correct overgrown beak and claws. Provide hard food items to promote beak wear (cuttlefish)</td>
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<td>Petechia / bleeding</td>
<td>Thrombocytopenia; Sepsis; Warfarin intoxication</td>
<td>Petechial haemorrhaging and ecchymoses, mainly on the ventral body surface and mucus membranes</td>
<td>History and clinical signs; Haematology &amp; biochemistry; blood smear</td>
<td>Antibiotics to treat sepsis. Vitamin K in case of warfarin toxicity</td>
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<td>Septicaemic cutaneous ulcerative disease (SCUD)</td>
<td><em>Citrobacter freundii</em>, but also other Gram negative bacteria (e.g. <em>Serratia</em>) Other factors implicated are poor husbandry, poor water quality, abrasions and invertebrate predation; mainly in aquatic and soft shell turtles</td>
<td>Irregular, caseated, crateriform ulcers on the plastron, carapax and skin. Secondary septicaemia; organ failure, haemolysis, limb paralysis and loss of digits or claws.</td>
<td>History; Inspection of vivarium; Clinical signs; Culture and sensitivity</td>
<td>Debridement of ulcers and abscesses; use of antibiotics; shell support with fiberglass and resin if destruction is extensive. Prognosis is usually poor if not treated promptly.</td>
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<td>(Subcutaneous swelling: Nodule)</td>
<td>Neoplasia (e.g. fibrosarcoma, papilloma); Bacterial or fungal granuloma; abscess (<em>Dermatophilus congolense</em> in bearded dragons); Calcinosis circumscripta (CC, due to underlying renal disease?); Endoparasites (cestodes, skin filariids)</td>
<td>CC: chalky appearance gritty consistency; Discrete, well-circumscribed subcutaneous swelling, may be hard of soft in consistency</td>
<td>History and clinical signs; Inspection of vivarium; Culture and sensitivity</td>
<td>Surgical excision in case of neoplasia or migrating endoparasites; Systemic antibiosis in case of infection. Renal failure may benefit from fluid therapy and sucralfate to reduce the uptake from phosphorus. Allopurinol is used in case of gout.</td>
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<td>(Subcutaneous swelling: Abscess (fibriscess))</td>
<td>Arises as a sequela to trauma (e.g. bite wound) or following haematogenous spread; Large range of (Gram-negative) bacteria and occasionally fungi may be involved</td>
<td>Raised, hard, well-circumscribed subcutaneous swelling, containing caseous pus. Most frequently seen on head and extremities. May involve bone and lead to systemic disease</td>
<td>Clinical aspect; FNAB; Culture &amp; sensitivity</td>
<td>Surgical removal of the abscess including the fibrous capsule. Lancing and flushing may be required if excision is not possible. Appropriate antibiosis based on sensitivity (preferably antibiotic with gram-negative spectrum)</td>
<td>Histology reveals a central core containing bacteria surrounded by a fibrous capsule.</td>
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<td>Shell deformities</td>
<td>Malnutrition (e.g. lack of Ca and Vit D) Excess protein High humidity</td>
<td>Hunchbacked shell; Dents and thickening of the carapax; abnormal shaped carapax and/or plastron, soft shell</td>
<td>History and clinical examination (soft shell); Radiographs are advised when metabolic bone disease or bone damage is suspected</td>
<td>Correct diet and husbandry; Deformities are permanent</td>
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<tr>
<td>Shell fracture</td>
<td>Trauma due to predator attack, fall injury, etc.</td>
<td>Fissures, fractures of the caparax and/or plastron; Paresis / paralysis may occur when dorsal aspect of carapax is broken</td>
<td>Clinical presentation; Radiographs to assess extent of the fracture and localization of potential fissures</td>
<td>Remove devitalized tissue Align and restore anatomy using cerclage and epoxy resin, dental or hoof acrylis and/or fiberglass mesh Infected wounds may initially be treated with bandages and topical and/or systemic antibiotics prior to attempting to repair the shell.</td>
<td>Healing may take 1-2 years; in young, growing chelonians grooves should regularly be made along the margins of individual scales</td>
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<td>Wounds (bite wounds)</td>
<td>Attack from predators; Feeding life prey</td>
<td>Wounds across the body surface or shell</td>
<td>History and clinical signs Culture and sensitivity</td>
<td>Cleaning, debridement and wound closure similar to burn wounds; Systemic (and/or topical) antibiosis</td>
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</table>
Selected References