Welcome to the 9th World Congress of Veterinary Dermatology. In the face of the unprecedented COVID-19 pandemic, we hope you, your family, friends, colleagues, students and clients have remained healthy and safe.

A major goal of the World Congress is to disseminate information on current diagnostic and treatment modalities in the various fields within our discipline. Despite the many changes to the typical program format, the exciting advanced and comprehensive Continuing Education Program highlights current trends in clinical dermatology practice from around the world. This information will be of value to general practitioners interested in veterinary dermatology as well as the specialists in the discipline.

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TABLE OF CONTENTS

DERMATOLOGY IN GENERAL PRACTICE

Pruritis and Infections
Diagnostic Approach to the Pruritic Dog – How to Avoid Missing Things Without Overservicing.......................... 1

Ralf Mueller

Staphylococcal Pyoderma – An Update on Diagnosis and Management................................................................. 4

Douglas J. DeBoer

Parasitic Skin Diseases in the Age of Isoxazolines ................................................................................................. 8

Ralf Mueller

Atopic Dermatitis
Clinical Signs and Diagnosis of Canine Atopic Dermatitis ..................................................................................... 12

Peter B. Hill

Canine Atopic Dermatitis -- Where do the New Treatments Fit In?................................................................. 15

Wayne Rosenkrantz

Allergen-Specific Immunotherapy -- Does it Still Have a Role? ................................................................. 23

Douglas J. DeBoer

Topical Therapy of Atopic Dermatitis ..................................................................................................................... 26

Meng K. Siak

Diagnostics and Therapeutics
Food and the Skin – Choosing the Right Diet ...................................................................................................................... 28

(Where Do Home-Cooked, Hydrolysed, Grain-Free BARF Diets and Support Diets Fit In?)

Ralf Mueller

Cytology in Dermatology Cases – What Does It Tell Us and How Can We Use It? ............................................. 33

Kimberly S. Coyner

Skin Diseases of the Muzzle and Nasal Planum ............................................................................................................. 37

Ralf Mueller

Otitis
Pathogenesis of Otitis Externa ........................................................................................................................................... 44

Craig E. Griffin

Diagnostic Approach to Otitis Externa ............................................................................................................................... 47

Peter B. Hill

Management of Acute Otitis Externa ......................................................................................................................... 49

Craig E. Griffin

Management of Chronic Ear Infections ....................................................................................................................... 53

Peter B. Hill

Alopecia and Endocrine Disorders
General Diagnostic Approach to Alopecias – Folliculitis versus Follicular Arrest ...................................................... 60

Monika M. Welle

Demodicosis – Is it Still a Problem? ............................................................................................................................... 67

Ralf Mueller

Dermatophytosis in Dogs – Update on Diagnosis and Treatment .................................................................................. 71

Deborah L. Simpson

Hypothyroidism – What Should We Be Doing? ................................................................................................................. 77

Catherine A. Outerbridge

Hyperadrenocorticism – What Should We Be Doing? .................................................................................................... 84

Richard A. Squires
Challenging Diagnoses
General Diagnostic Approach to Nodular Skin Diseases -- Is it Infectious, Sterile or Neoplastic? ...................... 92
Verena K. Affolter
Update on Flea Borne Zoonosis – What You Can Catch at Work ................................................................. 96
Michael R. Lappin
### ADVANCED DERMATOLOGY IN CLINICAL PRACTICE

#### Atopic Dermatitis
- The Genetics of Canine Atopic Dermatitis ................................................................. 101
  
  *Tim Nuttall*
- Update on the Immunopathogenesis of Canine Atopic Dermatitis ........................................ 107
  
  *Rosanna Marsella*
- Should Atopic Dermatitis be Redefined and the Diagnostic Approach Amended? .......... 111
  
  *Tim Nuttall*
- Allergy Testing – Is It Still Worth Doing? ........................................................................ 118
  
  *Douglas J. DeBoer*
- Management of Barrier Function – Does it Help? ............................................................... 120
  
  *Rosanna Marsella*

#### Genetics and Scaling
- Genodermatoses in Dogs and Cats .................................................................................. 123
  
  *Elizabeth A. Mauldin*
- A Stepwise Approach to Scaling Disorders ......................................................................... 124
  
  *Elizabeth A. Mauldin*
- Metabolic and Paraneoplastic Skin Disorders in Dogs and Cats ........................................ 128
  
  *Elizabeth A. Mauldin*

#### Diagnosis and Treatment
- Lasers in Veterinary Dermatology .................................................................................... 129
  
  *Michael A. Shipstone*
- Biological Therapies in Dermatology ................................................................................ 135
  
  *Douglas J. DeBoer*

#### Microbial Resistance
- Biofilms and Their Implications for Clinical Practice in Veterinary Dermatology .......... 138
  
  *Douglas J. DeBoer*
- Management of Resistant Staphylococcal Infections in Dogs – The Australian Perspective .... 141
  
  *David C. Robson*

#### Autoimmune Diseases
- A Practitioner’s Approach to Immune-mediated Skin Diseases ........................................ 151
  
  *Peter B. Hill*
- An Update on Cutaneous Lupus Erythematosus .................................................................... 157
  
  *John H. C. Hutt*
- Lokivetmab – Does it Do More Than Treat Atopic Itch? ...................................................... 161
  
  *Greg Burton*
- Advanced Immunosuppressive Therapy .............................................................................. 165
  
  *Wayne Rosenkrantz*
- Oclacitinib – Can it Do More Than Treat Atopic Dermatitis? .............................................. 174
  
  *Greg Burton and Mark Walczak*

#### Unusual Skin Lesions
- Adverse Drug Reactions in Dogs and Cats ........................................................................ 181
  
  *Stephen D. White*
- The Approach to an Unusual Skin Lesion or Disease ......................................................... 185
  
  *Peter B. Hill*
FELINE DERMATOLOGY

Itchy Cats
General Diagnostic Approach to Pruritic Skin Disease in Cats .......................................................... 188
Danny W. Scott
Allergy Testing in Cats – Is it Worth it? ........................................................................................................ 189
Amanda Burrows
Management of the Atopic Cat – Have we Moved Away from Prednisolone? ........................................ 195
Wayne Rosenkrantz
Pyoderma in Cats – Is it Really a Problem? ......................................................................................... 201
Danny W. Scott
Dermatoses of the Face, Head and Neck – How Often is it Food Allergy? ........................................... 203
Linda J. Vogelnest
The Eosinophilic Granuloma Complex – Has Anything Changed? ....................................................... 212
Danny W. Scott
Feline Otitis – Is it Different Compared to Dogs? ................................................................................. 214
David C. Robson

Feline Skin Lesions
Ventral Alopecia – Is it Dermatological, Behavioral or Medical? ......................................................... 225
Danny W. Scott
Dermatophytosis Treatment – What Works? ......................................................................................... 227
Kimberly S. Coyner
Pododermatitis In Cats ......................................................................................................................... 234
Danny W. Scott
Nodular Skin Diseases in Cats ............................................................................................................. 235
Meng K. Siak

Systemic Disease
Cutaneous Manifestation of Systemic Disease in Cats ........................................................................ 238
Linda J. Vogelnest
Feline Cushing’s Disease ......................................................................................................................... 246
Catherine A. Outerbridge

Autoimmunity
Autoimmune Skin Diseases in Cats ......................................................................................................... 252
Danny W. Scott
EQUINE DERMATOLOGY

Itchy Horses
Approach to the Pruritic Horse ...................................................................................... 254
Danny W. Scott
Management of Allergic Horses .................................................................................... 256
Rosanna Marsella
Culicoides Hypersensitivity – Future Prospects for Diagnosis and Treatment .................. 260
Eliane Marti, Antonia Fettelschoss, Sigridur Jonsdottir

Equine Skin Lesions
Differential Diagnosis of Follicular Dermatoses in the Horse .......................................... 264
Danny W. Scott
Approach to Pastern Dermatitis in Horses ....................................................................... 266
Rosanna Marsella
Skin Diseases in Donkeys ................................................................................................. 270
Stephen D. White

WILDLIFE AND EXOTIC DERMATOLOGY

Skin Diseases of Small Mammals .................................................................................... 274
Joanna Hedley
Sarcoptic Mange in Wombats and Koalas ...................................................................... 275
Scott Carver
Common Dermatologic Disorders in Pet Birds ................................................................. 279
Yvonne R.A. van Zeeland
Skin Diseases of Reptiles and Amphibians ..................................................................... 286
Patrick J. Bourdeau
Leprosy in Eurasian Red Squirrels ................................................................................... 287
Anna L. Meredith and Anna Katarina Schilling
Can We Save the Tasmanian Devil from Extinction? ......................................................... 293
Katherine Belov
Introduction
Pruritus may manifest itself as many clinical signs, including licking, rubbing and biting. Pruritus is a hallmark of many different diseases although allergies and ectoparasites are the most common. Typically if secondary lesions such as papules, pustules, crusts or scales are present, they are either due to self-trauma or to secondary infections. The list of differential diagnoses is long and the results of both the history and physical examination will determine the number and sequence of diagnostic tests recommended.

History and physical examination
The list of possible differential diagnoses for pruritic skin diseases is determined in part by the physical presentation. Non-lesional pruritus, also known as pruritus sine materia, is most likely due to allergies. However, ectoparasites may also be responsible and secondary infections with bacteria or yeast can be associated with lesions so subtle they are overlooked. In older animals, paraneoplastic pruritus or pruritus due to liver disease or azotemia may occur rarely. In cats with self-induced alopecia or hypotrichosis, psychogenic alopecia is a rare differential diagnosis and if the licking is focused on the abdomen or inguinal area, idiopathic cystitis should be considered.

If lesions are present, differential diagnoses depend on the type of lesions. For example follicular papules or pustules are most commonly due to either bacterial folliculitis, dermatophytosis or demodicosis. Large pustules spanning several follicles suggest either pemphigus foliaceus or bullous impetigo. The latter is a bacterial infection that in older dogs is frequently secondary to hyperadrenocorticism. In puppies, bullous impetigo is a transient condition in which topical skin barrier appropriate therapy should be pursued and not systemic antibiotics.

This list of differential diagnoses should be prioritized in order to choose the relevant diagnostic tests. Prioritization depends on the signalment and history of each particular patient. Very young patients tend to get infections, while young or young adult animals are presented with allergic disease, hormonal imbalances or metabolic disturbances. Older animals are more prone to develop neoplastic or immune-mediated diseases whereby pruritus is uncommon. Gender or breed predispositions need to be considered.

Seasonal disease points to involvement of fleas, chiggers or seasonal allergens. Onset of pruritus after changing the diet or after feeding specific food sources is compatible with food allergy. Non-seasonal pruritus may be associated with environmental allergens such as house dust mite or food allergy. In the case of non-seasonal pruritus always offer an elimination diet trial. Other clinical signs such as polyuria/polydipsia point to possible underlying systemic diseases such as hyperadrenocorticism. Some diseases are characterized by a sudden onset of pruritus (such as scabies or food allergy) while others tend to develop more gradually (such as environmentally-induced atopic dermatitis). Severity of pruritus may give some clues: severe pruritus refers to a
level at which the owners cannot sleep and the animal stops eating or playing to scratch itself. This is often associated with scabies. Mild-to-moderate pruritus is frequently seen in atopic dermatitis or infections. When pruritus persists following the successful therapy of any identified secondary infections and/or ectoparasite diagnostic therapy, then consider allergies as the most likely underlying cause of the pruritus.

Previous tests may have been conducted and considered suitable to rule out a specific disease. For example, a serum thyroid hormone concentration in the upper normal range rules out hypothyroidism in an older patient with *Malassezia* dermatitis. Such tests do not need to be repeated, as they most likely will not yield a different result. In contrast, other tests such as skin cytology may show bacterial infection and one month later with similar pruritus no bacteria, but yeast organisms. Such tests should be performed frequently to monitor response to therapy. The classic situation would be a dog scratching its ears with a diagnosis of a severe bacterial otitis externa, in which case cytology may be needed every couple of weeks for several months.

Historical response to previous treatments may give clinical clues about the underlying disease, although care needs to be taken to interpret the answer of the owner correctly. For example, the owners may state that there is no response whatsoever to antibiotics. This may indicate that administration of antibiotics does not change the clinical signs (interpreted as either an infection with resistant bacteria or no infection at all). But the owners may also mean that clinical signs resolve each time, only to recur within days after cessation of antibiotic therapy (interpreted completely differently — a bacterial infection is responding to treatment, but either a prominent underlying disease or incomplete treatment of the pyoderma leads to quick recurrence of infection after therapy ends).

**Further diagnostic testing**

Once history and physical examination have resulted in a prioritized list of differential diagnoses, there are, in general, three options to proceed further and I offer all three to my clients. The option more frequently recommended in specialist referral practice is what I refer to as “the Mercedes option”. All tests are performed that are needed to rule out or confirm most, if not all, diseases on the differential diagnoses list. In a six-year-old, male West Highland white terrier with moderate to severe pruritus, erythema, papules, lichenification, hypotrichosis and alopecia predominantly affecting the trunk this may include deep skin scrapings for demodicosis, superficial scrapings for the superficial mites, cytology to evaluate secondary infections, complete blood count, serum biochemistry and urinalysis, as well as, a thyroid assay to check for hormonal disease, good ectoparasite control, an elimination diet trial and possibly a skin biopsy to rule out epitheliotropic lymphoma. At the other end of the spectrum would be what I call “the bicycle option”, which is choosing to rule out or confirm the one or two most likely diagnoses. In the above described West Highland white terrier this may be ectoparasite control (particularly if the dog is not already on a good ectoparasiticide control program) and skin cytology to assess possible secondary infection. Of course, as the name indicates, this is a much less costly and less invasive option. The owner can also be offered a third in-between option, (“Ford option”), the extent of which depends on the owner’s psyche, owner finances, my own gut feelings and the number and type of differential diagnoses. I present those options to the owners and let them choose the one they would prefer. The advantage of the “Mercedes option” is that the diagnosis is achieved in the fastest way possible but also typically with significant cost and most likely a
number of tests not needed (because the “bicycle option” would be acceptable in the majority of patients). The “bicycle option” is usually the least invasive and always the least expensive. However, there may be a wait for the results of first-line of tests to come back before the next likely diagnosis is approached (the procedure may be repeated numerous times). For most patients, this approach will provide the diagnosis with the least cost involved. For the few patients with a rare and unlikely disease, this option may quite a while to get to the final diagnosis.

If owners make the decision themselves about how to approach the pruritus, there is the least chance of complaints and the highest chance of client satisfaction. It is important to remember that over-servicing for one client is great service for the other. One person may be very happy ruling out unlikely diseases for a considerable amount of money and will consider the “bicycle option” as ‘bad medical practice’ (particularly if the dog ends up with an unusual and less likely disease and the first tests do not reveal the diagnosis). Another owner may complain about one inexpensive additional test and call it over-servicing. It is all in the eyes of the beholder ….

Some tests may be more cost-effective than others (a deep skin scraping is much cheaper than a biopsy to diagnose demodicosis), while others such as many in-house tests (skin scrapings, cytology, etc.) may have the advantage that results are immediately available. Thus, a “bicycle option” (such as cytology and scrapings) can be done and then move on to the next level of diagnostics during the same visit, if the quick in-house tests have not rendered a diagnosis.

In summary:

1. Take a careful history and conduct a thorough clinical examination.
2. Determine the level of pruritus and the exact clinical signs and locations of pruritus.
3. Formulate a differential diagnosis list based on the above.
4. Always look for a secondary infection component and consider ectoparasite control as part of the diagnostic work-up for pruritus.
5. Remember laboratory serum tests for scabies and food allergies are not necessarily diagnostic, as false positives are associated with these tests.
6. Consider an elimination diet trial in a case with non-seasonal pruritus without secondary infections.
7. Be transparent—offer your clients the two or three levels of possible diagnostic work-up with an appropriate cost-benefit analysis.
STAPHYLOCOCCAL PYODERMA –
AN UPDATE ON DIAGNOSIS AND MANAGEMENT

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Clinical Features and Diagnosis
Staphylococcal pyoderma is an extremely common contributor to skin disease, especially in dogs. It has a variety of associated clinical presentations, which are often divided into surface, superficial and deep varieties. Most canine pyodermas are of the superficial variety and are characterized by papules, pustules, focal crusting, epidermal collarettes, “moth-eaten” alopecia and pruritus. Deep pyoderma can be recognized by the presence of furuncles and draining tracts.

Diagnosis of pyoderma is generally based upon physical examination, as lesions are very characteristic. When possible, cytologic specimens from intact or recently ruptured pustular areas will often demonstrate presence of coccoid bacteria. Procedures such as bacterial culture and skin biopsy are reserved for recurrent or resistant cases.

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” (IRSP), there are several measures that may help to prevent or limit recurrence.

The Complication of Antibiotic Resistance
Many years of empirical, indiscriminate use of systemic antibiotics for canine skin infections has led to a further complication — development of antimicrobial resistance. 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 (C/S) should be strongly considered. 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 multiple drug resistance becomes more likely with each cycle of treatment.

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 or S. schleiferi (i.e., the canine strains) 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.

**Primary Patient Treatments**

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, non-antibiotic treatments, if possible, especially in the face of recurrent infections. Increasingly, dermatologists understand that it is very possible to eliminate active superficial staphylococcal infections (even MRS) from the skin by using topical products as the primary treatment, without antibiotics. For primary topical treatment of an existing superficial pyoderma, *daily* treatment is necessary until the infection is cleared, which typically takes 4 weeks or more. Antimicrobial topicals are also the first line of defense for prevention of relapse in patients with recurrent pyoderma where the cause cannot be found or treated. For preventive maintenance, topicals are typically used 2-3 times weekly.

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.

Chlorhexidine (2-4% spray, mousse, gel, conditioner, etc.) is the best-studied topical antimicrobial ingredient for canine pyoderma. Studies suggest the concentration, at least within this range, is not terribly important. The specific formulation may be more important, as other ingredients within the shampoo can augment effectiveness and/or affect lathering ability or other qualities important for compliance. Combination with an antifungal ingredient such as
miconazole, clotrimazole or ketoconazole is often recommended, in no small part because *Malassezia* yeast overgrowth is frequently part of the clinical picture as well.

Clearly, there are situations where use of systemic antibiotics is still a preferred method of treatment. The most obvious example is deep pyoderma, where topical products simply cannot penetrate into the deeper soft tissue infection. Extensive or especially severe superficial pyoderma may also warrant systemic antibiotics. An important consideration here is antibiotic selection. Antibiotics for staphylococcal pyoderma should always be selected according to a logical scheme, which is often guided by antimicrobial susceptibility testing. One such scheme, devised by an international expert panel and based on study evidence, divides antibiotics into three “tiers” as follows:

**Tier 1.** For primary empirical treatment of known or suspected superficial pyoderma, the drugs of choice are either first-generation cephalosporins (cephalexin, cefadroxil); amoxicillin-clavulanate; or clindamycin/lincomycin. Potentiated sulfa drugs (e.g., trimethoprim-sulfa) can be added to this list if regional susceptibility patterns indicate these drugs are effective or if individual patient C/S indicates susceptibility.

**Tier 1/2.** Drugs for which there is insufficient evidence, and lack of consensus, about classifying them as Tier 1 vs. 2: this includes third-generation cephalosporins such as cefovecin and cefpodoxime. The controversy here is that some authorities believe that use of these antibiotics may have the effect of selecting for resistant strains of pathogens such as *E. coli*.

**Tier 2.** When empirical Tier 1 therapy and/or topical therapy are not appropriate AND based on C/S testing, the following antibiotics may be useful: doxycycline/minocycline; chloramphenicol; fluoroquinolones; rifampicin combinations; or aminoglycosides. The emphasis here is that C/S testing is crucial to antibiotic selection!

**Tier 3.** When Tier 1 and Tier 2 drugs are not appropriate, and culture indicates susceptibility: linezolid, teicoplanin, vancomycin. Use of these three antibiotics is *strongly discouraged*. These drugs can be considered “reserved for the treatment of serious MRSA infection in human beings.”

**Treating Recurrent Infections**

As outlined above, managing recurrent infections should first center on correction of any identifiable underlying cause and on frequent, intermittent use of topical antimicrobials. 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.
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.

**Sanitation, Awareness, and Public Health**

MRS is spreading very fast in both the human and veterinary worlds. It is time to start taking every precaution to prevent transmission of MRSP strains to other pets in your clinic and to prevent colonization of in-contact people with MRSA. Key measures for sanitation have been developed by expert panels and include handwashing and disinfection; wearing gloves when appropriate; protective clothing, with frequent laundering; cleaning and disinfection of premises, especially exam and waiting room areas; and education of staff and pet owners.
PARASITIC SKIN DISEASES IN THE AGE OF ISOXAZOLINES

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Introduction
In the past, ectoparasite control was tailored to the specific parasites and situation. As a consequence, flea control was often conducted with different therapeutic agents than, for example, the treatment of scabies, and successful medications for scabies were not necessarily efficacious against Demodex mites. With the advent of isoxazolines, which are effective insecticides and acaricides, the question arises: is this drug class the ‘panacea’ and will it replace all other medications? Or will there still be a need for alternatives?

Isoxazolines
Indications. Currently in Europe, the registered isoxazolines include fluralaner (Bravecto®), afoxolaner (Nexgard®), sarolaner (Simparica®) and lotilaner (Credelio®). Fluralaner is administered every three months, while the other three are monthly medications. All were initially registered as flea and tick control agents. Fluralaner has persistent tick killing activity for 12 weeks against Ixodes ricinus, Dermacentor reticulatus and D. variabilis, and persistent tick killing activity for 8 weeks for Rhipicephalus sanguineus. Afoxolaner and lotilaner have persistent tick activity for 4 weeks against Dermacentor reticulatus, Ixodes ricinus, Ixodes hexagonus and Rhipicephalus sanguineus. Sarolaner is approved against those ticks for 5 weeks. Fluralaner, afoxolaner and sarolaner are also registered as treatment for canine scabies and demodicosis; sarolaner is additionally registered as a treatment for Otodectes cynotis. Fluralaner and afoxolaner are approved for animals heavier than 2 kg body weight; sarolaner and lotilaner heavier than 1.3 kg. For all four isoxazolines, animals should to be older than 8 weeks when treated.

Adverse effects. Mild gastrointestinal adverse effects have been observed with all four products in a small number of patients and typically resolve without further treatment. Systemic signs such as lethargy and anorexia, and neurological signs such as muscle tremors, ataxia and convulsions have been very rarely reported with fluralaner, afoxolaner and sarolaner. It is advised on the package insert to use fluralaner cautiously in patients with epilepsy. However, overall those products have a very good safety profile.

Scientific evidence of isoxazoline efficacy
There are numerous studies published and only selected references are shown at the end of the notes. Here is a summary of the studies.

Flea control. A large number of studies in dogs have shown the efficacy of fluralaner, afoxolaner, sarolaner and lotilaner for the control of flea infestations in the dog and for fluralaner in the cat. The efficacy at various time points (2, 4, 8 and 12 weeks post administration) was in general >99%. In laboratory studies, fluralaner killed 8% of the fleas after 1 h, 37%, 88% and >98% after 2, 4, and 8 hours respectively. In one comparative study, the flea kill count at 6h was significantly higher with afoxolaner (46-98%) than with fluralaner (64-100%); there was no difference at all other time points. In another comparative study, sarolaner had a significantly
higher flea kill count at 8h compared to fluralaner; no difference was seen at any other time point. A third study compared afoxolaner and sarolaner and evaluated the flea kill count after 8, 12 and 24 h, with sarolaner being more effective after 8 and 12 h, particularly several weeks after administration. However, as in the previous studies, there was no difference at the 24h time point. In reports evaluating privately owned dogs, clinical signs of allergic dermatitis resolved in 80-100% of the dogs receiving fluralaner, afoxolaner and sarolaner. In one comparative study, fluralaner and afoxolaner both improved signs of flea allergy by >80%. Similar results were seen in dogs and cats using topical fluralaner. In one study, fluralaner prevented the transmission of Dipylidium caninum by fleas. In summary, all oral isoxazolines show a very good efficacy against flea infestations in the dog and for topical fluralaner this also is true for cats. Oral afoxolaner has also been shown to be effective in one study in cats.

Tick control. Afoxolaner, fluralaner, sarolaner and lotilaner have been reported to be close to 100% effective for Ixodes scapularis, Dermacentor reticulatus, Ixodes ricinus, Rhipicephalus sanguineus infestation. The killing efficacy of fluralaner against Ixodes ricinus was 90% after 4h, 98% after 8h and 100% after 12 h. Percent efficacy against Dermacentor reticulatus at 24h post-infestation for afoxolaner and fluralaner ranged from 85.2% to 99.6% and from 63.4% to 99.1% respectively. Fluralaner treatment efficacy against Ixodes holocyclus was 100% at 72 h post treatment, lotilaner and sarolaner were also effective. Oral treatments with fluralaner, sarolaner and afoxolaner had an 8 h speed of kill (>90% efficacy) threshold for Rhipicephalus sanguineus, while fluralaner and sarolaner achieved 100% efficacy at 12, 24 and 48 h; afoxolaner achieved 100% efficacy at 48 h. Monthly oral doses of sarolaner provided > 95 % efficacy within 24 h of treatment, and consistently provided > 70 % efficacy against subsequent re-infestations with Amblyomma americanum within 24 h. A single treatment of fluralaner spot-on provided 100% efficacy against I. holocyclus ticks for at least 84 days in cats.

As ticks can transmit a number of pathogens, the question arises whether isoxazolines kill ticks rapidly enough to prevent a transmission of such pathogens. In three studies, sarolaner, lotilaner and afoxolaner prevented the transmission of Babesia canis by ticks; in another study, sarolaner prevented transmission of Anaplasma phagocytophilum. Further studies showed the ability of afoxolaner and sarolaner to prevent the transmission of Borrelia burgdorferi. In contrast, neither afoxolaner nor fluralaner could prevent transmission of Ehrlichia canis to dogs from Rhipicephalus sanguineus.

Treatment of demodicosis. Sarolaner eliminated Demodex mites in dogs in one South African study. In another small study, lotilaner eliminated all Demodex mites within one month in 9/10 dogs and after three months all dogs were in remission. In a larger study in referral practice, the administration of afoxolaner monthly to dogs with generalized demodicosis led to negative skin scrapings in 75% of dogs after three months and 12 dogs (25%) had an average mite count of <4 mites per scraping. Approximately 80% of the dogs were in clinical remission after the three months. A large study evaluated fluralaner for the treatment of generalized demodicosis in 115 dogs, all of which were in remission after 4 months.

Treatment of scabies. In several studies, fluralaner (both orally and topically), afoxolaner and sarolaner eliminated scabies mites and improved clinical signs in all dogs.
Treatment of ear mites. In two studies, one administration of fluralaner completely eliminated ear mites in 47 affected cats (topical) and 16 dogs (topical or oral). Sarolaner decreased ear mites in 179 dogs by >98% in another couple of studies. Afoxolaner eliminated ear mites from cats and dogs in two studies.

What are the limitations of isoxazolines?

Expense and availability. Isoxazolines are costlier than some other ectoparasiticides and not all owners may be willing to regularly spend money for ectoparasite control with those agents. In fact, due to expense, the author has observed a number of owners using these drugs on an “as needed” basis. This has two potential drawbacks. The first, with respect to flea control, is that intermittent treatment of fleas has been associated with the development of flea allergic dermatitis. The second is with respect to tick control – failure of the owners to visualize ticks may allow transmission of an infectious disease or development of tick paralysis.

Repellant action. Although there is evidence that afoxolaner kills sand flies, isoxazolines have no repellant activity and thus transmission of leishmania and other pathogens is possible. Isoxazolines are rapid-onset insecticides, and transmission of Babesia canis or Borrelia burgdorferi, can be prevented (see above). However, for other pathogens, studies have either not been conducted or data were less convincing.

Patient limitations. Some dogs or cats may be sensitive to isoxazolines and show enough adverse effects to prevent their future use in those individual patients.

Resistance. Although to the author’s knowledge no isoxazoline resistance has been conclusively documented in the literature, anecdotal evidence of resistance to individual products exists. Whether the lack of resistance is a true resistance against the class of drugs, if it is due to a lack of absorption in some patients or if other factors are involved, is currently not known.

Selected References

6. Dryden MW, Canfield MS, Kalosy K et al. Evaluation of fluralaner and afoxolaner treatments to control flea populations, reduce pruritus and minimize dermatologic lesions in naturally


CLINICAL SIGNS AND DIAGNOSIS OF CANINE ATOPIC DERMATITIS

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Two recent publications provide detailed guidelines on the approach to the diagnosis of canine atopic dermatitis. The guidelines published by the Australian Veterinary Dermatology Advisory Panel provide a comprehensive overview of the diagnosis and management of pruritus in dogs. These guidelines can be obtained from the following URL: https://www2.zoetis.com.au/academy/dermatology. Another freely available publication “Canine atopic dermatitis: detailed guidelines for diagnosis and allergen identification” can be obtained from the open access journal BMC Veterinary Research. Both of these publications outline a thorough and logical approach that can easily be accommodated in the general practice environment.

The first step in the diagnosis of any skin disease is to obtain a detailed history, but this can be challenging in the limited time available in a typical general practice consultation. One way of dealing with this dilemma is to use a questionnaire that can be emailed to the clients prior to the consultation. In the past, this recommendation was not widely embraced by general practitioners, but an easy-to-use, editable PDF version of such a questionnaire can make life a lot easier. Such a questionnaire can be obtained from the Vets Australia website (https://www.zoetis.com.au/vets-australia). Use of such a questionnaire not only ensures consistency of history taking; it provides a complete record that can be added directly to the animal’s file.

The key historical features that typify the phenotype of canine atopic dermatitis are a pruritic skin disease that usually starts between 6 months and 3 years of age (but occasionally later). The pruritus tends to occur at typical distribution sites such as the face, ears, paws, axillae, ventral chest and abdomen and perineum. Pruritus can manifest in a number of different ways including scratching, rubbing, chewing, excessive grooming, rolling, scooting, and/or head shaking. The pruritus can be seasonal in some cases, but in the majority it occurs throughout the whole year. In an individual case, there can be considerable variation from the classic pattern described above. For example, some dogs may present solely as foot chewers, face rubbers or ear scratchers. Alternatively, some dogs have more widespread pruritus that affects the rest of the trunk.

Careful physical examination is critical when assessing patients with dermatological problems in order to assess the lesion types, distribution and severity of the condition. Allergic dermatitis is often a progressive disease with gradually worsening lesions. Unlike parasitic or infectious skin disease, allergic dermatitis can present in the early stages with no (or very subtle) skin lesions. Subsequently, the skin will become inflamed resulting in a dermatitis. The dermatitis is usually regional but diffuse in nature, compared to the papular eruptions seen with parasitic or bacterial skin disease. If the condition becomes more severe, secondary skin lesions can develop such as self-induced alopecia and seborrhea. It is common for atopic skin to become secondarily infected due to disruptions in the normal microbiome. This will result in additional skin lesions associated with staphylococcal infection or Malassezia overgrowth. With chronicity, some breeds (especially the West Highland white terrier) will develop lichenification and hyperpigmentation.
of their skin. The point at which a patient is at along this pathological continuum is important because it determines how difficult the patient will be to treat and which treatment options are likely to be required in order to reverse the pathology.

The distribution of skin lesions in dogs with atopic dermatitis often follows a broadly similar pattern, but there can be individual variations. Two recent studies have also demonstrated that there can be subtle differences in the lesion distribution between breeds. In a large study originating from Switzerland\(^1\), some of the breed-specific differences observed were:

- German shepherd dogs were highly likely to have lesions on the ventral abdomen
- Shar Peis had a higher risk of lesions over the dorsum, lateral trunk and lateral hind limbs
- West Highland white terriers had a higher risk of developing lesions on the dorsum
- French bulldogs had a high risk of developing lesions in the axillae

In a similar study undertaken in Australia\(^2\), some of the findings included:

- The Kelpie was less likely to have lesions on the medial pinnae
- The German shepherd dog, boxer, West Highland white terrier and Maltese terrier were more likely to have lesions over the dorsum
- The boxer, Kelpie and beagle were highly likely to have lesions on the ventral chest

One study has attempted to determine the statistical probability of various clinical criteria and lesion distributions to help with the diagnosis of atopic dermatitis. Known as “Favrot’s criteria”\(^3\) these provide a set of signs that yield reasonably high sensitivity and specificity for the condition. However, the lack of complete accuracy due to the variation between individual patients means that these have not been widely adopted in clinical practice.

Due to the lack of pathognomonic signs, when examining a case of suspected atopic dermatitis it is important to follow a logical diagnostic approach in order to establish a definitive diagnosis. The first step in this approach is to rule out conditions with clinical signs that can resemble or overlap with canine atopic dermatitis. While this is traditionally referred to as a “work-up” it is important to remember that a “one size fits all” standardised work-up is not always necessary. Clinical judgement and experience should always play a part in determining what types of tests are appropriate in a particular case.

As a general rule, the following steps should be performed before arriving at a diagnosis of atopic dermatitis:

1. Consider the possibility of fleas. The likelihood of fleas being involved in a pruritic skin disease varies dramatically in different parts of the world. In areas where fleas are present, clinicians should rule them out based on lesion distribution, physical examination, flea combing and/or an effective flea control program.
2. Rule out other ectoparasites such as *Sarcoptes, Demodex, Cheyletiella, Otodectes* and *Trombicula*.
3. Check for staphylococcal and *Malassezia* infections. This can be done based on clinical signs and cytology.
4. Consider the role of adverse food reactions. If client compliance can be achieved, a 6 week exclusion diet using an appropriate food should be undertaken.
If the above steps are followed and the dog has appropriate historical and clinical features, a diagnosis of atopic dermatitis can be made. At that stage, the clinician has the choice of initiating symptomatic treatment or investigating the condition further using allergy tests. Two methods of allergy testing are routinely available for the further investigation of canine atopic dermatitis: intradermal skin testing and in-vitro measurement of allergen-specific IgE (IgE serology). Intradermal skin testing is typically carried out at dermatology referral centers, whereas IgE serology can be carried out in general practice. To date, there is no evidence that clearly indicates that one test is superior to the other. Also, it is well known that the results of the two tests do not completely agree when performed in the same patient. Furthermore, the degree of disagreement is somewhat dependent on the specific antigen that is tested and the center at which the tests are performed. Additional details about allergy testing are found in other notes of the Congress.

Whether or not an allergy test should be performed depends on a number of factors including the severity of the pruritus, age of the dog, financial considerations and what the owner wishes for their pet. Although allergy testing should not be relied upon to make a diagnosis of atopic dermatitis, it does provide two additional benefits that may be of interest to owners. First, it can identify what a patient is allergic to, which some owners simply want to know. However, it should be made clear to owners that allergen avoidance strategies after a positive allergy test are unlikely to provide any real benefits for the majority of atop ic dogs. Second, allergy tests provide the opportunity for allergen specific immunotherapy (ASIT), which can be a valuable treatment option for some dogs. Additional details about ASIT are found in other notes of the Congress.

Selected References
Introduction
Ciclosporin was originally used as an immunosuppressive agent, primarily to prevent organ transplant rejection but the drug has been recognised as a highly effective treatment option for the management of pruritus associated with canine atopic dermatitis. It has over 10 years of pharmacovigilance in its use for the treatment of canine atopic dermatitis and has been shown to be a safe therapy. It is also highly effective and registered for treatment of feline atopic dermatitis. There are multiple reviews available for the veterinarian regarding the use of ciclosporin in the management of atopic dermatitis.

Oclacitinib maleate (Apoquel®, Zoetis) is a synthetic janus kinase (JAK) inhibitor developed for the treatment of allergic skin diseases in dogs. It has a high degree of efficacy, a rapid speed of onset and fewer adverse effects than corticosteroids. A compassionate use study demonstrated that oclacitinib was safe and efficacious for long-term use and improved the quality of life for dogs with atopic dermatitis. A pilot study also demonstrated encouraging results using oclacitinib for the management of feline allergic skin disease. However, the drug is not registered for use in cats. Cytopoint® (Zoetis) is the first monoclonal antibody (mAb) therapy approved for control of the clinical signs associated with atopic dermatitis in dogs. Cytopoint is an injectable product containing a caninized mAb specifically designed to target and neutralize cytokine interleukin-31 (IL-31). It works by mimicking the activity of natural antibodies to selectively bind and neutralize IL-31. Having these drugs with a comparably high degree of efficacy has been extremely beneficial for the management of atopic dermatitis (AD) in dogs and cats. The question of which drug to select for an individual patient will be based on many variables but should occur on a case-by-case basis. The following discussion will cover the advantages and disadvantages of each of the drugs.

Ciclosporin
Ciclosporin (CsA) inhibits intracellular calcineurin and is a potent inhibitor of T cell activation. Calcineurin is an enzyme involved in transmitting signals to the cell nucleus by dephosphorylating nuclear factor of activated T cells (NFAT). Blocking this prevents gene transcription necessary for production of multiple interleukins and cytokines, in particular IL-2 production. The effects of CsA in atopic dermatitis may be more complex as a beneficial response can be seen with low serum levels that are not associated with IL-2 reduction. CsA does affect canine keratinocytes and may affect cutaneous dendritic cells, innate immunity as well as inhibit mast cell degranulation by affecting the interaction between mast cells and nerves. CsA is metabolised by the cytochrome P450 enzyme system, specifically CYP3A4, in the liver and intestines. Drugs that inhibit CYP3A4 or compete with P-glycoprotein may therefore substantially alter CsA metabolism and cytochrome P-450 inducers such as phenobarbital and rifampicin may decrease CsA levels. P-450 inhibitors may increase CsA levels as well. This includes antifungal azole drugs (ketoconazole, itraconazole, fluconazole), furosemide and a number of calcium channel blockers. However, not all of these drugs inhibit the specific hepatic microsomal cytochrome P-450 (CYP) 3A12 enzyme. Ketoconazole represents one important and
A useful drug interaction as it suppresses the cytochrome P450 enzyme system, thereby decreasing CsA clearance and increasing CsA blood concentration.

Atopica® (Elanco) is the only approved veterinary microemulsion formulation that improves absorption of the drug and is available in capsules of 10mg, 25mg, 50 mg and 100mg. A liquid formulation is also available (Atopica for Cats®, Elanco) at 100mg/ml. The absorption of CsA can be variable depending upon the formulation; Atopica and Neoral® (the human form similar to Atopica, Novartis) are more readily absorbed than its predecessor Sandimmune® (Novartis). Atopica is most consistently absorbed when given without food on an empty stomach, although in some dogs the incidence of vomiting and other gastrointestinal adverse effects may be reduced by administering the drug with food.

A high incidence of nephrotoxicity and hepatic toxicity is reported in people receiving CsA, but this is not commonly observed in dogs. In a review, adverse events (AEs) have been reported in 55% of 759 dogs receiving CsA in 15 clinical trials but are rare in pharmacovigilance data. Gastrointestinal reactions were most common but mild and rarely required intervention. Other AEs were rare (<1 per cent in clinical trials; <10/million capsules sold). Hirsutism, gingival overgrowth and hyperplastic dermatitis were rarely significant and resolved on dose reduction. CsA decreases staphylococcal and Malassezia infections in AD and at the recommended dose is not a risk factor for other infections, neoplasia, renal failure or hypertension. Concomitant treatment with most drugs is safe. Effects on cytochrome P450 and MDR1 P-glycoprotein activity may elevate plasma CsA concentrations, but short-term changes are not clinically significant. At the authors’ practices, the incidence of vomiting in dogs is approximately 35%, with most cases responding to either a dosage reduction, dividing the dose or giving the drug with food, although this can reduce effective serum levels. A variety of products to control vomiting have been recommended, including metoclopramide (Maxolon®, Amdipharm) 0.1 to 0.5 mg/kg q 12hrs and maropitant citrate(Cerenia®, Pfizer) 2 mg/kg q 24hrs. When CsA is combined with ketoconazole, there is an increased risk of both these side effects.

Azithromycin administered orally and topically in a toothpaste formulation may be of value in the management of gingival overgrowth in people and dogs. Papillomatosis has been reported in a limited number of cases. There can be an increased risk of infections, especially viral. In human beings, there is an overall increased risk of cancer with use of CsA. Increased risk of skin neoplasia, particularly squamous cell carcinoma, and increased incidence of lymphoma in humans may be associated with a higher incidence of Epstein Barr virus infections. Lymphoma in dogs has not been substantiated and there is a report that showed no correlation between CsA for use in AD and development of mycosis fungoides and leukemia. The author has observed cases of multiple plasmacytomas in a single dog and histiocytomas arising in dogs during CsA therapy that have resolved with either dosage reduction or discontinuation of the drug.

Opportunistic bacterial and fungal infections can arise secondary to immunosuppression from CsA therapy administered for the management of atopic dermatitis. Cutaneous and systemic infections with Nocardia species and Mycobacterium species have been reported. Rare cases of systemic atypical deep mycotic infections such as Prototheca, Aspergillosis and Phaeohyphomycosis species have also been observed. However, these typically arise in organ transplant patients receiving concurrent potent immunosuppressive drugs such as high dose
glucocorticoids. Side effects to CsA in cats are considered rare. Concerns have arisen about increased susceptibility to viral and other latent infections. Fatal acute systemic toxoplasmosis has been diagnosed in a cat after 8 months of CsA therapy for feline atopic syndrome. Young male cats are predisposed and hunting and feeding raw meat are risk factors. Clinical toxoplasmosis most commonly arises within four to eight weeks after onset of therapy, especially in cats with high CsA blood concentrations.

Monitoring serum trough levels of CsA is a controversial issue. In the authors’ experience the serum levels of CsA do not usually correlate with the clinical outcome and thus the routine monitoring of serum peak or trough levels of CsA in the stable canine or feline patient is not recommended. There remains debate, however regarding the significance of CsA serum levels and the frequency of adverse reactions, and there are several reports suggesting that high trough concentrations (700 to 1000ng/ml) in cats and 400 to 600ng/ml or more in dogs may be more frequently associated with side effects. The author considers measuring serum trough levels in situations where the clinical response to CsA is poor and a failure of drug absorption is suspected. Levels in the low therapeutic range (<200ng/ml) could warrant an increase in the dose rate. Conversely, performing serum trough levels is recommended if there are signs of a possible adverse health event that may be associated with CsA or concurrent administration of a drug that may affect the CsA concentration (e.g., ketoconazole or itraconazole). With respect to monitoring for medium to long-term side effects, the author routinely performs pre-CsA lab work and monitor cases every 6 to 12 month with complete blood counts, serum chemistry profiles and urinalyses. There is a low incidence of bacteriuria in dogs receiving CsA. However, many dogs that have developed bacteriuria or cystitis were previously receiving glucocorticoid therapy. There have also been reports that renal enzyme levels (BUN and creatinine) may elevate in dogs on chronic therapy but there are limited reports of nephrotoxicity. When used as a long-term therapy, patients should be monitored for the development of atypical infections and neoplasia.

Oclacitinib
Oclacitinib maleate (Apoquel) is a drug developed for the treatment of allergic diseases in dogs. It is a synthetic drug of a relatively new class of drugs called Janus kinase (JAK) inhibitors. Janus kinases are a group of four enzymes [Janus kinase 1-3 and tyrosine kinase (TK)] that function by facilitating the first step in transmitting intracellular signals, enabling a cytokine that has bound and activated a cell surface receptor to have an effect on that cell. They function in pairs with certain cytokine receptors being acted on by various combinations of paired enzymes such as JAK 1 and 3, or JAK 2 and 3, or JAK 3 and TK. Oclacitinib acts predominantly to inhibit JAK 1 and at higher serum levels to inhibit JAK 2. The Cmax of the drug relates to the dose and the 90% confidence interval for the Cmax at a dose of 0.6mg/kg administered daily for 168 days is 273-406 ng/ml. The Cmax at a dose of 0.4mg/kg is approximately 200ng/ml for beagle and mongrel dogs after a single dose. The cytokines most sensitive to JAK 1 enzyme inhibition from the highest to the lowest are IL-31, IL-2, IL-13, IL-14 and IL-6. At much higher oclacitinib serum levels, other JAK 1 and JAK 2 sensitive cytokines including erythropoietin (EPO) and granulocyte and macrophage colony stimulating factor (GM-CSF) are affected. This drug has been considered an immmodulator because its most potent effect is blocking the activity of IL-31, which controls pruritus activity.
Efficacy has been demonstrated in the management of pruritus associated with allergic disease in many studies. A blinded placebo-controlled study evaluated oclacitinib in 436 dogs with a variety of allergic diseases. Pruritus scores decreased from 7.39 to 2.59 and 7.58 to 5.54 in the oclacitinib and placebo treated groups respectively. The response in the treated group was significantly better than the placebo group. Another blinded placebo study performed by board certified veterinary dermatologists evaluated oclacitinib for the management of atopic dermatitis in 299 dogs. Pruritus scores decreased from 7.8 to 2.6 and from 7.7 to 7.4 at the 14-day scoring in the oclacitinib and placebo treated groups respectively, with very significant differences between groups. All dogs participated in a subsequent open label study and at the end of 112 days the pruritus score averaged 3.2. The skin lesions as graded by the canine atopic dermatitis extent and severity index (CADESI) reduced from a pre-treatment score of 62 to 32 and 58 to 57 in the oclacitinib and placebo groups respectively. When all dogs completed the open label phase, the CADESI was 26 at Day 112. Another study also demonstrated that oclacitinib has efficacy in the management of flea allergy dermatitis. Lastly, the author finds oclacitinib helpful to use at the beginning of dietary trials to aid in pruritus reduction until the dietary trial can be completed. The dose of oclacitinib for all allergic disease cases is 0.4 to 0.6mg/kg given 12hrs for 14 days then q 24hrs. It is available in 3.6, 5.4 and 16mg tablets. Although most dogs had pruritus controlled at once daily dosing, the author and other dermatologists have found that some dogs need their once daily dosing divided BID for control of pruritus. There are also dogs that require their induction dose to be maintained for long-term control. This is not routinely recommended as the incidence of adverse reactions could be more frequent at continued high dose therapy. However, the author and others have used induction dosing long term and rarely are complications seen with this regime.

Details regarding specific studies and frequency of adverse events can be reviewed in the Apoquel® Product Label information. The short-term safety profiles based on data from multiple randomized-controlled clinical trials is very good. In a 28-day study with 436 dogs, the incidence of diarrhea, vomiting, anorexia and polydipsia were low at 1.4-2.3%, which was similar to placebo-treated dogs (0-1.8%). In most cases, clinical signs resolved while continuing the drug. A long-term compassionate care study evaluated 247 client-owned dogs with allergic skin disease that had previously benefited from oclacitinib therapy. Owners completed a quality-of-life survey and assessed pruritus using a Visual Analog Scale (VAS) throughout the study and veterinarians recorded adverse events, concomitant medication and clinical pathology results were summarized. The percentage of dogs showing >/=50% reduction from baseline on day 90 was 63.9% for PVAS and 66.4% for CADESI. Owners saw a positive impact on quality of life in >91% of all dogs.

Urinary tract infection/cystitis (UTI), vomiting, otitis, pyoderma and diarrhea were the most frequently reported (>5% of dogs) abnormal clinical signs (UTI 11.3%, vomiting 10.1%, otitis 9.3%, pyoderma 9.3%, diarrhea 6.1%). Hematology and serum chemistry levels remained within the normal reference ranges. Concomitant medications were well tolerated. However, there were 16/247 dogs that did develop some type of confirmed or suspected malignant neoplasia of which 10 dogs were euthanized. The association with Apoquel and tumor formation in these cases is not known. A study performed at author’s practices looked at a comparison of malignancies and non-malignant skin masses in 339 allergic dogs receiving long-term (> 6 months) oclacitinib with age and breed matched control population. The incidence of malignancies in the oclacitinib
group (16.5%) versus controls (12.8%) was not statistically different (p=0.1742). The incidence of skin masses in the oclacitinib group (56.6%) versus controls (58.3%) was not statistically different (p=0.6743). The age of death/euthanasia in the oclacitinib group (11.2 years, n=80) versus controls (11.8 years, n=72) was not statistically different (p=0.2077). There was no statistical significance regarding the dose of oclacitinib on cumulative incidence of malignancy or masses.

Adverse effects have been reported in published and unpublished reports. UTIs incidence is variable with some reports showing no cases and others showing a low incidence of 6.5%. Anecdotal reports of weight gain have been in some patients on oclacitinib. Whether it is related to reduced pruritus or other causes has been proposed. The potential association of weight gain may reflect the impact of JAK-STAT transcription factors involved with adipocyte metabolism including insulin action, modulation of lipid stores, leptin levels, IL-6 and glucose homeostasis. In addition, there are also anecdotal reports of increased tendency for pyoderma, demodicosis and otitis externa also exist. The author typically avoids using Apoquel in dogs with any history of pre-existing neoplasia and in dogs with deep infection, history of demodicosis or in severely immunocompromised dogs. It is contraindicated for use in breeding dogs or pregnant/lactating bitches. It has been used in limited cases with concurrent low dose glucocorticoids or cyclosporine for short periods of time.

Apoquel is currently being evaluated for use in the allergic cat and some safety profile data is available from Zoetis. In addition, a controlled study showed good safety data with no side effects at dosages of 1-2 mg/kg q 12hrs in a 28-day treatment period. There are now a number of papers published using oclacitinib in the cat with variable results. It does appear that higher dosages are needed in cats as metabolism appears more rapid, many of the studies utilized dosages at 0.8–1.3mg/kg twice daily. The author recommends a minimum dosing of 1mg/kg q 12hrs for induction, dosing can be reduced in some cases to 0.5 mg/kg q 12hrs after the first 2 to 4 weeks.

The author currently performs baseline serum chemistry profiles, complete blood counts and urinalysis testing. This is repeated at 6-month intervals in patients on continuous therapy. Patients also need to be monitored for the development of clinical infections while on long term therapy.

There is one 84-day randomized controlled trial comparing Apoquel to Atopica in 226 dogs. Dogs were evaluated for PVAS and CADESI-02 scoring on days 1, 2, 7, 14, 28, 56 and 84 days. Differences were significant at all time points up to day 28 regarding PVAS scoring. Apoquel treated dogs had a more rapid onset of activity regarding the pruritus reduction. More adverse events were found in the Atopica treated group, which were predominately gastrointestinal in nature. Vomiting and diarrhea occurred in 44% and 15% respectively of the dogs in the Atopica group versus 14% and 4% respectively in the Apoquel group. A retrospective study compared the incidence of cutaneous histiocytoma development in atopic dogs treated with oclacitinib (n=533) versus ciclosporin (n=654). There were 14/533 and 4/654 patients who developed histiocytomas while on oclacitinib and ciclosporin, respectively. There was a significantly higher percentage of histiocytomas on oclacitinib (2.6%) versus ciclosporin (0.6%) (P=0.0041). However, in the previously mentioned study performed at the author’s practices comparing
malignancies and non-malignant skin masses in 339 allergic dogs receiving long-term (> 6 months) oclacitinib with age and breed matched control population, there was no statistical increase in histiocytomas in the Apoquel treated group.

**Cytopoint — Caninized Anti-cil-31 Monoclonal Antibody (Mab)**

Monoclonal antibodies are complex large protein molecules that are developed by utilizing recombinant DNA technology that mimics the natural immune response in the body. They are inactivated by digestion so are given by injection, not orally. They are used at monthly or longer intervals and are designed to have target specificity and minimal side-effects. They interact extracellularly with a free or cell surface target and cannot act intracellularly. They are degraded by normal protein catabolism to amino acids which are then reused by the body, with minimal hepatic or renal metabolism and elimination.

Cytopoint is a caninized anti-canine interleukin-31 (IL-31) monoclonal antibody. IL-31 has been shown to induce pruritus in dogs in laboratory studies. Lokivetmab is the active compound in Cytopoint. Lokivetmab has the advantage of being extremely targeted and having a very long half-life, remaining in circulation for several weeks. The label allows for repeated administration, monthly, or as needed. Initial experience suggests Cytopoint may be effective in some canine patients that had an insufficient response to other forms of allergy therapy, including cases that have failed oclacitinib therapy. In a clinical trial involving client-owned dogs with atopic dermatitis, a single injection of 2 mg/kg began to reduce pruritus within 1 day and was effective for a full month. On day 3, greater than 80% of dogs administered Cytopoint achieved treatment success (predefined as an owner-assessed ≥20-mm reduction in pruritus as scored on the pruritus Visual Analog Scale [VAS]). Mean pruritus scores were very mild starting at day 1, continuing for a full month. There was significantly greater efficacy vs placebo in achieving treatment success in improvement of skin lesion scores (predefined as a 50% reduction from baseline in veterinarian-assessed skin condition) beginning on day 14 and continuing for 1 month (p≤0.05). Significant improvement in skin condition was noted at the first visit on day 7. At day 28, there was a nearly 50% decrease in dermatologist scores for treated dogs compared to dogs receiving placebo. In this clinical trial, side effects were minimal, manageable and similar to placebo. The most common side effects were vomiting, diarrhea and lethargy. In another field safety study, Cytopoint was well tolerated in dogs after subcutaneous injection. Adverse events occurred at a similar frequency between treated and placebo groups in a study of 245 canine patients. Abnormal health events were self-limiting and not continuous through the length of the study. A wide variety of concomitant medications were safely used, including parasiticides, antibiotics, antifungals, corticosteroids, vaccines, immunotherapy, antihistamines and other antipruritics, such as oclacitinib and cyclosporine. Cytopoint has also been demonstrated to be well tolerated in a laboratory safety study in which 7 consecutive monthly subcutaneous injections were administered to laboratory beagle dogs at doses of 3.3 mg/kg or 10 mg/kg body weight (12 dogs per group).

In 37 dogs that were closely followed and evaluated at one of the author’s practices, 50% of the cases had good to excellent control, with 25% having a partial reduction in pruritus and 20% of the cases had no improvement. In the cases that showed responses, 25% had relief for less than 4 weeks. More repetitive, reduced interval of injections or higher dosing has been used in some cases, which can help dogs that do not initially respond well. In addition, recent Zoetis data
suggests that it may take up to 3 monthly injections before the full benefit on pruritus reduction can be seen. In some cases, the duration of control is longer than one month. This may reflect seasonal atopic dermatitis and individual case responses. In another report of 101 dogs, 73% responded well to treatment and 27% were considered non-responders. Responders received a mean of 4.6 injections and the mean time between injections in the group of responders was 34 days. A decrease in efficacy of treatment was not observed in a group of 32 dogs that received six or more injections. In a large multicentre clinical study, 181 veterinary clinics that purchased ≥20 vials of product were asked to record the dates for the duration of effect. For dogs that received more than one injection during the study, the mean treatment interval was 36.8 days (range, 12-101), 33.5 days (19-79), and 32.5 days (25-56), respectively, for dogs that received two (n=293), three (n=92), or four injections (n=18). Of the dogs that received two injections, 80.2% received the second dose between 28 and 56 days, 7.8% at >56 days, and 11.9% at 27 days. The mean treatment interval was 36.8 days. The impact of disease seasonality or concurrent therapy was not assessed.

The author uses Cytopoint commonly during the beginning of immunotherapy and at the start of diet trials, particularly in dogs less than one year of age. However, it may not be as good as oclacitinib during dietary trials due to its variable duration of activity. It can be used as a long-term monotherapy or as part of multimodal therapy in chronic atopic dermatitis cases. It appears very safe with minimal adverse reactions. An occasional dog may exhibit sensitivity or pain at the injection site and mild lethargy. In one review of 132 treated dogs, adverse events were limited and mild. They were reported in 11/132 (8.3%) dogs and included lethargy (8), vomiting (2), hyperexcitability (1), painful reaction at the injection site (1) and urinary incontinence. A rare anecdotal anaphylactic reaction has been described by a couple of clinicians, some requiring hospitalization. Reduced efficacy after months of therapy is also rare and most commonly is due to failure to control secondary infection or other flare factors. To date, the development of autoantibodies has not been reported to occur to any significant degree.

**Selected References**

ALLERGEN-SPECIFIC IMMUNOTHERAPY -- DOES IT STILL HAVE A ROLE?

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Allergen-specific immunotherapy (ASIT) is a familiar treatment for allergic diseases of dogs, cats and horses, wherein extracts of allergens to which the patient is sensitive are administered, in gradually increasing amounts, to lessen the hypersensitivity state. What’s new in this field and what can we look forward to in the future?

Does ASIT Still Have a Role in Management of Allergy?
With the recent advent of new (and sometimes wondrously effective) drug and biological treatments for allergy, some have questioned if ASIT is as necessary or useful as in the past. The author believes very strongly that ASIT should remain a foundational and important part of a multimodal treatment approach. It is the only treatment for allergy that can modify, or reverse, at least part of the pathogenesis of this condition as we know it – both alleviating clinical signs and preventing progression in the process. This modification is accomplished without the possible long-term adverse effects of a lifetime of drug treatment, with minimal chance for its own adverse effects, and with the potential of long-lasting effectiveness. There are certainly disadvantages to ASIT including the fact that it takes several months or more to begin working, that it does not always work and that it may be relatively expensive. Nevertheless, if we are to provide optimal management of this lifelong disease, ASIT still has a primary role.

As far as is known, concurrent treatments with antihistamines, fatty acid supplements, ciclosporin, oclacitinib, lokivetmab or even low-dose glucocorticoids will not interfere with response to ASIT. Such treatments are virtually always necessary as part of the overall treatment plan to provide immediate and short-term relief while waiting for the ASIT to work. These treatments can be slowly tapered as response to ASIT occurs. Treatment with ASIT is generally considered to be lifelong, though it is possible to attempt discontinuation after 2 to 3 years of injections if the animal has responded very well.

Injection versus Sublingual ASIT
Current ASIT approaches in atopic dermatitis (AD) center on either subcutaneous (SCIT) or sublingual (SLIT) administration. There are a great many unknowns about both methods and elucidating these may facilitate improvements in therapy. To date, there have been no studies in pets that directly compare the results of ASIT with injection versus sublingual administration; the very limited evidence available suggests both have approximately equal efficacy. In human beings, the choice is still an ongoing discussion.

Sublingual immunotherapy (SLIT) involves administration of allergen extract into the oral cavity, under the tongue, as opposed to by injection. It is commonly used for human allergy in Europe, particularly for atopic rhinitis and asthma. Historically, there are conflicting reports of efficacy, which may be explained in part by the extreme variation in protocols used for dosing, administration, intervals, vehicle, etc. in the different studies reported. Consideration of recent evidence has led authoritative bodies to conclude that, when used correctly, it is clearly
efficacious and in fact has a response rate similar to subcutaneous ASIT. Its use in animals is relatively new.

One big advantage of SLIT is in ease of administration, which may improve compliance. Although many owners “don’t mind” giving injections to their pets, most owners clearly don’t relish it and are delighted to be presented with an alternative to giving injections. Most dogs accept administration easily (even viewing it as a treat), which increases compliance. On the other hand, successful SLIT requires faithful twice-daily administration and owners with busy schedules may find it much more convenient to give an infrequent injection. In human beings, anaphylactic reactions to SLIT are rare to nonexistent and SLIT can be used in humans with a prior history of reaction to allergy shots. The same appears to be true for pets.

**Choosing, Mixing, and Dosing Allergen Extracts**

Protocols for ASIT in pets are completely unstandardized and subject to enormous variation. Different veterinary dermatologists are likely to use different allergen doses of unstandardized extracts that vary in composition and potency from manufacturer to manufacturer and from batch to batch, using different schedules of administration, differing concurrent treatments and with different ‘rules’ about how to mix extracts together. With the incredible variation among dermatologists in how, and how many, extracts of different types are mixed together, it is no wonder that patient experience varies. Should the number of extracts in a mixture be limited to eight? Ten? Twelve? Or unlimited? Should protease-containing extracts such as molds be admixed or administered only by separate injection? Should the mixture be based upon results of intradermal testing? Serologic testing? Both? Or merely made uniform for every patient, based on what allergens are predominant in a region?

On the human side, these debates have not ended, though recent large-scale studies are providing at least some guidance. Evidence is accumulating that in polysensitized human beings, treatment with a single, dominant allergen is as effective as multi-allergen ASIT, even though polysensitization is more prevalent. This “less is more” approach, prevalent in Europe, is unpopular in the United States among physician allergists. The evidence for limiting the number of allergen extracts used in treatment stands in contrast to protocols used by many veterinary dermatologists, and if applicable to dogs, will require a change in our thinking.

Most effects of ASIT are thought to be allergen-specific, rather than nonspecific. Thus, accurate testing to identify the offending allergens in each patient is of paramount importance to successful immunotherapy. In particular, the clinician must strive to avoid ‘false positive’ allergy test results, which would result in including an allergen in the patient’s mixture that is not relevant to that individual’s disease.

**ASIT in Cats and Horses**

Despite substantial clinical need, progress in the world of feline and equine ASIT has been limited. For cats, a big part of the problem is in defining “feline atopic disease” and how cases should be selected for ASIT treatment, and difficulties in interpreting allergy testing results in cats. In horses, some progress has been made in the realm of hypersensitivity to the biting midge *Culicoides*. 


For the Future: What Might We See?
Canine AD appears to have remarkable similarity to human atopic dermatitis, in that this allows us to examine new research findings in people for ideas about what might be useful in dogs. In addition, murine models of AD being used to reflect the situation in people probably also mirror that in dogs. Potentially important advances in human beings that have yet to be explored in dogs include: use of recombinant major allergens or allergen peptides; enhancing the effect of allergens using adjuvant-like manipulations such as IL-10 inducers; packaging allergen in virus-like particles (VLPs); or in the case of SLIT, mucoadhesive polymers. Co-administration of immunomodulators such as CpG oligodeoxynucleotides or specific monoclonal antibodies might “push” the immune response in the desired non-allergic direction, while reducing inflammatory mediators of active disease, thus allowing ASIT to work more effectively. And last but not least, combining any of the above with new methods of administration such as intralymphatic injection might be tremendously exciting in companion animals.
TOPICAL THERAPY OF ATOPIC DERMATITIS
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Introduction
Canine atopic dermatitis is defined as a genetically predisposed inflammatory and pruritic skin disease associated with well-defined clinical signs and immunoglobulin (IgE) directed against environmental allergens. In cats, feline allergic skin disease (FASD) is less understood. The severity of pruritus and extent of skin lesions varies between dogs and cats, and therefore there is no “one size fits all” management plan. Instead, an individualised plan should be made for each pet that involves both systemic and topical medications.

To successfully manage atopic dermatitis, the clinician needs to reduce the level of pruritus, treat secondary infections, control against reinfections and improve the skin barrier. Topical therapy plays an important role in achieving this. Most of the studies performed using topical therapy for atopic dermatitis have been on dogs. Topical therapy is usually used in adjunctive to systemic therapy. Topical therapy can only be effective if the dog or cat is receptive and owner is compliant.

Topical therapy: anti-pruritic
Topical corticosteroids and calcineurin inhibitors are effective as anti-pruritic agents, allow the reduction of systemic anti-pruritic required and therefore risks of potential side effects. Deciding which topical corticosteroid to use depends on factors including required potency, dose and how corticosteroid is applied and vehicle. Generally, a more potent corticosteroid is used to control the pruritus before switching to a lower potency corticosteroid to control against flares. Prolonged use of topical corticosteroids can result in identical side effects as seen with systemic use.

In dogs, topical triamcinolone acetonide (0.015%, Genesis, Virbac) and hydrocortisone aceponate (0.0584%, Cortavance, Virbac) have been showed to be effective. Proactive twice weekly applications of hydrocortisone aceponate (0.0584%, Cortavance, Virbac) can also help maintain remission and extend the time to flares for patients with atopic dermatitis and otitis. Similarly, topical 0.1% and 0.3% tacrolimus have been shown to be effective in managing canine atopic dermatitis. In cats, hydrocortisone aceponate (0.0584%, Cortavance, Virbac) is effective for feline allergic dermatitis.

Topical therapy: anti-microbial
Atopic dogs and cats are predisposed to recurring secondary bacterial and Malassezia infections. Topical antimicrobial therapy can be effective as a sole treatment against surface and superficial bacterial pyoderma regardless of methicillin susceptibility, as well as Malassezia dermatitis.

Benefits of topical antimicrobial therapy
- May avoid the need for systemic antibiotics
- More rapid resolution of infection
- Reduce duration of systemic antibiotics required
• Break up biofilms, remove crusts, debris, bacteria, Malassezia and allergens from the skin
• Control against reinfections when underlying disease is being investigated and managed
• Reduce environmental contamination
• Reduce risk of transmission to other dogs/cats and humans

The in vitro minimum inhibitory concentrations (MICs) for many topical anti-bacterial therapies against meticillin susceptible *Staphylococcus pseudintermedius* (MSSP) and meticillin resistant *Staphylococcus pseudintermedius* (MRSP) are low and likely exceeded by concentrations achieved with topical applications. Therefore, topical antibacterial effective against MSSP are also effective against MRSP. These include 2-3% chlorhexidine, benzoyl peroxide, miconazole, fusidic acid, mupirocin, triclosan, bacitracin and polymixin B. Topical antimicrobials are available in shampoos, conditioners, lotions and sprays for more generalized infections, and creams, lotions, gels and wipes for more localized infections. Generally, a minimum of contact time of 10 minutes for shampoos is recommended. Lotions/sprays/wipes can be used up to daily if required. Topical antibacterial therapy can have antibacterial residual effect for at least 10 days. Household bleach (sodium hypochlorite) can also be considered and in vitro studies show that this can be effective against MRSP at concentrations between 0.05% to 0.2%. This should be replaced daily as the efficacy decreases with storage. Note that different bleach products may have different concentrations of sodium hypochlorite. For *Malassezia* dermatitis, there is good evidence for using 2% chlorhexidine/2% miconazole shampoo twice weekly and 3% chlorhexidine shampoo.

**Topical therapy: improving skin barrier**
Atopic dogs have a skin barrier dysfunction (primary and secondary), allowing allergen penetration and sensitization, as well as microbiome dysbiosis resulting in recurring bacterial and *Malassezia* infections. Topical therapy aimed at restoring the barrier function can assist in controlling against reinfections, soothe the skin and reduce pruritus, and potentially help against sensitisation to new allergens. One of the most important way to achieve this is to hydrate the skin and reduce transepidermal water loss (TEWL).

**Selected References**
Introduction
Diet and the integument are closely interrelated and are implicated in many areas of small animal practice. The skin acts as a mirror of an animal’s general health and both systemic illnesses and nutritional deficiencies may first be recognised by changes in the haircoat and skin surface such as a dull coat, hairloss or scaling. Food allergy, a hypersensitivity to ingredients in the diet, is a common differential diagnosis for pruritic skin disease in dogs and cats. Additionally, the sale of commercial dog food is often a source of income for individual practices. Owners frequently consult us and ask for advice about the best diet for their pet’s skin disease as the choices can be bewildering and contradictory information is frequent. There are many commercial dry, canned/tinned, hydrolysed or selected protein diets, as well as commercial frozen diets based on raw materials for an owner to choose from and many breeders have special recommendations of home-cooked diets. In these notes, an overview of diet and food allergy and various diets with their pros and cons will be discussed, based on available evidence and my personal opinion.

Diets for healthy dogs
In many countries, commercial dry or canned food, when it bears the statement ‘suitable as exclusive nutrition’ for dogs and cats must contain a recommended minimum requirement of protein, carbohydrates, fat, minerals and vitamins and thus should be sufficient to keep our pets healthy for a long time. However, these minimal requirements are not necessarily optimal concentrations (those for the most part are unknown and difficult to determine) which may also vary with breed, age and minor health changes. In addition, the bioavailability of the ingredients can vary widely, as was demonstrated decades ago with the so called “generic dog food” zinc deficiency disease. A number of dog and cat foods are marketed as “high quality” diets and this definition may or may not reflect reality, but typically is reflected by the price and meat content. Home-cooked and BARF (“Bones and Raw Food” but now frequently called “Biologically Appropriate Raw Food) diets are other options.

Commercial dry and canned food. In many countries, most owners feed commercial dog foods for convenience reasons. They are typically nutritionally balanced and thus suitable for long term use without any supplementation. Whether diets for specific breeds are indeed beneficial, has not been conclusively shown in randomized control trials to the author’s knowledge. However, young and growing animals will have different requirements from grown pets and, as in humans, extensive physical activity will increase the needed food intake. In contrast to many humans, dogs should not have a problem with being fed the same or a similar food every day and healthy dogs do not benefit from selected protein diets. Those diets should be reserved for dogs with food allergies. The author considers feeding hydrolysed diets to healthy dogs unnecessary.

Commercial Bones and Raw Food (BARF). People who feed their dogs raw food do so for a multitude of reasons, including but not limited to: culture, beliefs surrounding health, nutrition
and what is perceived to be ‘a more natural diet’ for their pets. For convenience reasons, many owners feeding BARF choose prepared commercial frozen diets. Commercial BARF diets are presumed to be nutritionally balanced, however over-supplementation and deficiencies of nutrients have been reported, especially regarding calcium, the trace elements copper, zinc and iodine, vitamins A and D and the calcium:phosphorus ratio. In addition, there is concern about the contamination of BARF diets with human and canine pathogens. In a number of studies, 20-80% of commercial chicken-based BARF diets were contaminated with *Salmonella* species. In other studies, 100% of BARF diets tested contained *Escherichia coli* of which 23-80% were resistant to extended spectrum cephalosporins. In addition, *Listeria monocytogenes* was found in more than half of the diets tested. On the upside, in contrast to dry food (which are associated with lower creatine intake), the creatine content of BARF diets corresponds to natural food sources. If owners decide to use BARF diets, careful food hygiene is of particular importance.

**Diets for itchy dogs**

There are a number of studies looking at various commercial diets for amelioration of non-specific pruritus in dogs. Most of those are characterized by a higher amount of essential fatty acids. In one open study, pruritus in 50% of the pruritic dogs was controlled with a diet enriched with fatty acids. In randomized, controlled and blinded trials, the effects were less pronounced but still statistically significant in many studies.

**Food allergy and elimination diets**

*Clinical signs raising suspicion for the presence of a food allergy.* In dogs, adverse food reactions can occur at any age but the mean age of disease onset in various studies is between one and four years. In one-third to one-half of dogs, clinical signs begin before one year of age. This predisposition of young age was confirmed in a recent study where the percentage of dogs with environmental atopic dermatitis and an onset of less than one year was significantly smaller (16%) than that of dogs with food-induced atopic dermatitis (48%). Importantly, offending food allergens may have been part of the diet one to two years before onset of disease. Pruritus is typically longer standing and non-seasonal, as most pets are fed the same diet all year round. Gastrointestinal signs, atopic dermatitis and angioedema have been reported in association with food antigens. In cats, the cutaneous reaction patterns of miliary dermatitis, head and neck pruritus, noninflammatory alopecia and eosinophilic granuloma complex are all recognized with food allergy.

*Diagnosis.* There are numerous studies showing that serum testing for food-specific IgE is poorly specific. The use of these tests as a diagnostic tool are a waste of money. Although negative results in some tests may be slightly more reliable than chance, none of the tests (not even the recent Western blots) reliably identify a clinically relevant food adverse reaction. There are only two studies evaluating intradermal testing and their results are identical — there was no correlation between results and reality. Testing the hairs and saliva of affected animals has been advertised by many companies as an option to diagnose adverse food reaction in small animals. There are a couple of studies looking at salivary testing with results being as unreliable as serum testing; these tests are not recommended by the author. Only one study evaluated testing of hairs to diagnose food allergy and the results were also disappointing. In that study, canine IgE was successfully measured by the laboratory in question from the synthetic hairs of a toy animal (which is impossible).
Patch testing, using Finn chambers, has been reported in dogs where raw and cooked meats and carbohydrate sources were the representative food allergens. The test was characterised by a high negative predictability and negative likelihood ratio. Thus, a negative reaction makes an adverse food reaction to this particular antigen very unlikely. However, the positive predictability was not very good and a positive result was difficult to interpret as some dogs were (based on provocation) actually allergic to these antigens, whereas others tolerated the antigens without clinical problems. Those findings were confirmed in subsequent studies. Patch testing with protein sources seems to be more reliable than with carbohydrate sources. This test is time consuming and requires three visits. It is most indicated in those dogs where it is difficult to choose a novel protein, because of a previously widely varied diet. Ingredients of the elimination diets can then be chosen from allergens that did not elicit a reaction on the patch test. Commercial dry foods were also used for patch testing in these studies, but the results were unsatisfactory (possibly due to the lower concentration of the individual allergens in the kibbles). Japanese studies have evaluated incubating lymphocytes of affected patients with various food antigens and compared the proliferation rates with lymphocytes stimulated with concanavalin (positive control) and non-stimulated lymphocytes (negative control). In those studies, the results were very encouraging. However, the lymphocytes need to be incubated immediately after obtaining the blood sample, the tests are technically not easy and the technique is currently not commercially available.

In summary, the above-mentioned tests are either not recommended or are not for common use. Feeding an elimination diet is still the best way to diagnose adverse food reaction. The only test the author recommends, in rare individual patients, is the patch test, which is then used to identify the protein source for an elimination diet trial.

Elimination diet. For an owner to perform a home-cooked elimination diet is hard work in most cases. The veterinarian also invests considerable time in educating the owners in the methodology - including how to nutritionally balance a non-commercial diet, continuously monitoring response to and encouraging compliance throughout the 6 to 8 weeks or longer needed to make the diagnosis. The owner must source ingredients, maintain food ingredient isolation and cook, which for many is time consuming. They may additionally battle with palatability issues, especially in cats. An elimination diet for canine patients consists of a protein source and a carbohydrate source. These are home-cooked and usually fed in a ratio of three parts of the carbohydrates and one part of the protein. Possible proteins are rabbit, horse or kangaroo, but any protein source previously not fed is suitable. Similarly, carbohydrate sources may be rice, potato, sweet potato, kidney beans, tofu or yam. The protein part can be increased, but should not fall below 20-25%. Nothing else is included in the diet. In cats, only the protein source is necessary on a short-term basis, as most cats are not extremely eager to eat rice, potatoes or beans. While it is ideal for the food sources to have never been fed previously, one may need to choose a food which has been fed less frequently. If owners fed treats previously, treat options should be offered for them to continue these habits without violating the overall diet. Depending on the diet, jerky of the used meat, rice cakes, potato chips or little pieces of fried or grilled meat are all possibilities to allow the feeding of snacks without adding additional protein sources. An elimination diet should be started gradually by adding small amounts of the new food to the normal food. This holds particularly true for cats. In dogs, some dermatologists recommend short-term fasting to increase acceptance and response time. If pets still do not like
the food, spices such as salt may increase palatability. Warming the food in the oven or microwave may also increase acceptance.

Commercial elimination diets with unusual proteins/carbohydrates (rabbit, duck, venison, kangaroo, potato, oats, etc.) or hydrolyzed proteins are available on the market. In hydrolyzed diets, protein size is decreased by hydrolysis to decrease or abolish allergenicity of the protein. In humans, the most common hydrolyzed proteins are milk proteins. In pet food, hydrolyzed soy and chicken products are currently marketed. It is important to remember that commercial diets will not cause remission in all patients with food adverse reactions and are thus only the second-best option! In our clinic, we propose home-cooked diets. If owners chose commercial diets and we see no improvement and subsequent intradermal testing or testing for serum allergen-specific IgE is unsatisfactory, we again propose a home-cooked diet to rule out food adverse reaction! As recent studies have shown most selected protein diets to be contaminated by other food sources (e.g., a duck and tapioca diet also contained mammalian and fish proteins), we recommend extensively hydrolysed diets as commercial alternatives of home-cooked elimination diets for the diagnosis of adverse food reactions.

**Re-challenge.** After a period of time, the patient will either be in remission, partial remission or will not have improved. This may take 6 to 8 weeks or longer before the patient is finally reevaluated. The time period can be decreased if prednisolone or oclacitinib are administered for two weeks initially to improve clinical signs. If after cessation of the medications clinical signs stay in remission for the following two weeks, a provocation may be initiated after four weeks. It is important that concurrent secondary infections are managed appropriately and that follow-up examinations or at the very least telephone calls are conducted to assess ongoing response. Physical visits can also be used to monitor the diet compliance. The aim is that once the animal is in remission, or a steady-state of partial but significant improvement, a re-challenge with the previously fed diet is performed. If there is significant improvement, the diet can be continued for another 6 to 8 weeks to evaluate the full degree of possible improvement on the diet. However, if this proves difficult for the owner, re-challenge may be indicated right away.

Assessing response to the diet is based on either a complete lack of improvement after 8 weeks or longer or else the return of the clinical signs within a few days or at the most 2 weeks on the old diet provocation. If the signs do not return on provocation, food adverse reaction is ruled out. Ideally, in a case where provocation does lead to a return of clinical signs, a sequential re-challenge with individual proteins is started to identify the offending protein. In our practice, we feed beef mince, then lamb mince, then chicken, then add cheese or milk in the diet for dairy products (if previously fed) and finally pasta to check the wheat proteins. Each protein is fed for approximately one week (which may vary if the individual took longer to react during the provocation with the original/old diet). A guestimate of the time until deterioration can be achieved by how quickly the symptoms returned after the previous re-challenge. If there was deterioration within 2 days, it is very likely that this pattern will be repeated. In best practice, once the offending protein is identified, it is avoided in the future. Most dogs and cats react to only a few proteins in our experience, even though some animals deteriorate on several different ones. However, other dermatologists feel they frequently see food adverse reactions involving many allergens. Some owners are happy with their pet’s clinical remission and the diagnosis based on first provocation and refuse to perform the sequential re-challenge. In these cases, it is
impossible to know the causative protein and several commercial diets containing a limited number of, or unusual, or hydrolyzed proteins can be tried. If no deterioration occurs, these foods are continued. If the home-cooked diet is chosen as a permanent solution, it needs to be balanced, preferably by a nutritionist, to avoid long term problems.

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CYTOLOGY IN DERMATOLOGY CASES – WHAT DOES IT TELL US AND HOW CAN WE USE IT?

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Introduction and indications for in-house cytology
Dermatologic diseases are one of the most common reasons dogs and cats are presented for veterinary care, and microscopy enables us to provide the accurate diagnoses, appropriate treatment and monitoring of cases to ensure they are responding to treatment satisfactorily. In the majority of cases, cytology can be performed in the clinic efficiently and economically, enhancing patient care and generating income.

Get a good microscope!
Buying a good microscope is essential to enable accurate cytologic examinations; fortunately, excellent biological laboratory microscopes can easily be obtained online for economical prices ($1000-2000). Some microscopes have small pop-up screens for teaching or to demonstrate findings to pet owners. A microscope pays for itself quickly: if the in-house cytology charge for a typical pyoderma or otitis patient is $25-50 x 5 patients per day = $125-250/day x 5 days per week = $625-1250/week. Use the old clinic microscope for fecal examinations and keep the new microscope dedicated to cytology only. It’s important to educate ourselves and our veterinary technicians on how to keep the microscope clean and in working order; there are many convenient online resources.

Cytology of inflammatory skin disease
Cytologic examination of inflammatory skin diseases is important to answer several questions:

- Is there infection and, if so, are the infectious organisms cocci bacteria, rod bacteria, yeast or a combination? This impacts choice of oral and topical medications.
- Are there bacteria present despite empiric antibiotics to make a bacterial culture indicated?
- Is the case not responding to antibiotics because of suspected bacterial resistance, a new bacterial population or Malassezia infection?
- Is the case not responding to antibiotics because it is not a bacterial disease? Malassezia dermatitis and dermatophytosis can be clinically similar to a bacterial pyoderma and non-infectious diseases such as pemphigus foliaceus, sebaceous adenitis and cutaneous lymphoma can mimic pyoderma.
- What kind of inflammatory cells or other cells are present?

Additional considerations:

- Prior to culture, cytology is important to decide if culture is warranted. For example, if only Malassezia and no bacteria are seen on cytology, then culture is not indicated.
- Prior to culture, cytology is important to be able to interpret culture results. If cocci were seen cytologically but only rod bacteria are grown on culture, ask the lab to recheck the results and consider repeating the culture.
- If no bacteria or yeast are found on cytology, this may indicate the need for dermatophyte culture or biopsies for non-infectious causes of dermatitis such as immune-mediated disease, keratinization disorder or cutaneous lymphoma.
- Cytology is also important to perform prior to biopsies because infection will alter histopathology results and mask the primary disease. For example, mucocutaneous pyoderma and discoid lupus are identical on pathology and bacterial pyoderma can be a cause for acantholysis and be falsely interpreted as pemphigus. Bacteria are sometimes difficult to see on biopsies and lack of bacteria on histopathology does not mean infection is absent.

**How to obtain samples for cytology analysis**

Cytology samples of skin lesions can be obtained in a number of ways:

- Rupture a pustule: the edge of the slide or a needle can be used to rupture the pustule then the pustule contents are smeared onto the microscope slide.
- Impression smear of moist or oily areas: the microscope slide can be directly pressed onto the area of interest; this is a good technique for axillary/inguinal and ventral neck dermatitis and to obtain samples of exudate under crusts after using the slide edge to gently move the crust over. For interdigital areas, use your finger to push the interdigital web onto the slide.
- Dry scrape and schmear: for areas of dryer scaling or crusting, a dulled scalpel blade with no mineral oil can be gently scraped over the area to collect debris which is then “schmeared” onto the microscope slide.
- Tape prep: use clear acetate (not frosted) tape and press firmly onto the area of interest; the tape can then be stained as with a slide or placed directly on top of a drop of methylene blue stain (Diff Quik #3) onto a microscope slide.
- Label each slide to ensure you know the site sampled and to indicate when side of the slide the sample is on, so you don’t wipe it off during drying.
- Heat fixing is not usually needed and can damage cells, the methanol (Diff Quik #1) is the fixative.
- Place the slide in each component of the Diff Quik stain for 30 seconds, then gently rinse the slide to remove excess stain and allow to dry/gently blot dry.
- Scan the slide under 10x magnification to identify an area of interest with numerous cells/staining to assess, then examine the cells and infectious organisms under 40-100x magnification.

**Interpretation of skin and ear cytology**

*Normal vs. abnormal:* When assessing slides obtained from inflammatory skin or ear conditions, it is important to know what is normal:

- For skin cytology, < 2 of each type of organism (cocci bacteria, rod bacteria or yeast) per oil immersion field (OIF) is normal.
- For otic cytology, < 3 yeast/OIF in dogs and < 1 yeast/OIF in cats may be considered normal; < 5 cocci bacteria/OIF and < 1 rod/OIF is considered normal.
- Inflammatory cells are not found on normal skin or ear cytology.
- Intracellular bacteria are not needed to make a diagnosis of a pyoderma; ≥5 extracellular bacteria/OIF = bacterial overgrowth.
- *Simonsiella* bacteria are huge rod-shaped normal oral flora commonly found on lips and paws; they do not indicate infection but can be a clue that the dog is licking the affected area.
- Pollen grains and saprophytic fungal spores are also commonly found.
• https://www.aaaai.org/about-aaaai/newsroom/photo-gallery/photos-graphics-pollen is a great website for pollen photos.

• Melanin granules, keratohyalin granules, and stain precipitate can mimic bacteria cytologically.
  o Melanin granules are brown and often refractile.
  o Keratohyaline granules are pink variably shaped globules within epithelial cells; they contain filaggrin and other proteins important for epidermal structure and function.
  o Stain precipitate is irregularly shaped, often amorphous purple debris; changing stains every 1-2 weeks and keeping fecal stains separate from skin cytology stains can help reduce the chance of stain precipitate, debris, or dead fecal bacteria mimicking infection on skin cytology slides.

Non-bacterial infections
Non-bacterial infectious organisms, which can be found on skin surface cytology include dermatophyte arthroconidia and hyphae, Candida, deep fungal infections and Leishmania.
• Dermatophytes do not form macroconidia on the skin, only in culture.

Inflammatory cell infiltrate
It is also important to assess the inflammatory cells on cytology of skin lesions, as the type of inflammatory cells found can be a clue as to the cause of the dermatitis:
• Neutrophils are commonly seen with pyoderma or self-trauma but can also be seen in immune-mediated diseases.
• Acantholytic cells are basophilic large round nucleated epithelial cells from the deep epithelial layers of the skin and can occur on skin cytology due to deep bacterial or dermatophyte infection (due to enzymes produced by the organisms which damage the intercellular epithelial connections) or to pemphigus foliaceus (due to immune attack of intercellular epithelial connections).
• Pyogranulomatous inflammation can be seen with chronic deep pyoderma as well as dermatophytosis and if found on cytology from a draining tract can indicate deep bacterial or fungal infection, foreign body, or sterile panniculitis.
• Eosinophilic inflammation can be seen with hypersensitivity to insects or other allergens, including canine eosinophilic facial furunculosis and feline eosinophilic plaques/granulomas, or can sometimes indicate an immune mediated disease or drug reaction.
• Neoplastic lymphocytes may be found on cytology of a scaly itchy dog with cutaneous epitheliotropic lymphoma.

Conclusions
• Teach your technicians to perform and interpret skin and ear cytology samples so they stay interested and you can see more patients.
• Post learning pictures to Veterinary Information Network (VIN) and send samples from questionable cases to a reference lab for pathology review.
The more cytology you do, the better and faster you will become. Performing in-house cytology is good medicine, which allows us to better diagnose and treat our patients, and generates more revenue!
Diseases of the nose and muzzle are typically in one of three categories: they are either caused by immune-mediated disease, infection or neoplasia. As always in veterinary dermatology, history and physical examination will give crucial clues to the differential diagnoses and determine the further choice of diagnostic procedures.

**History**
A thorough history will provide clinical clues. In old animals, neoplasia and immune-mediated disease are more commonly seen. In very young animals, infections are more prevalent. Previous treatments or tests may point to certain disease groups. Previous response to antibiotics is indicative of mucocutaneous pyoderma.

**Physical examination**
Physical examination will identify other parts of the body that are involved. In patients with lupus erythematosus, depigmentation of the planum nasale and lesions in the periocular area and on the dorsal muzzle are common. Generalized involvement may be seen with epitheliotrophic T-cell lymphoma. Severe lethargy and depression as well as hyperkeratotic foot pads are common in metabolic epidermal necrosis. Erythema multiforme may be accompanied by erythematous lesions in unusual shapes and forms elsewhere on the body.

**Diagnostic tests**
Depending on the differential diagnoses, cytology, blood biochemistry, urinalysis and complete blood count or biopsy may be considered. Cytology of the lesions on nose and muzzle is typically obtained by impression smears. If severe crusting is present, it is often very useful to “peel off” the crust and obtain an impression smear of the underlying erosion. Staining with Diffquick is sufficient — numerous yeast organisms or inflammatory cells with intracellular organisms are diagnostic of infection and acantholytic cells are suggestive of pemphigus. If cytology is not diagnostic for infection or if it is considered secondary to an immune-mediated process, a biopsy is strongly recommended. Biopsy is be needed for immune-mediated diseases such as pemphigus and bullous pemphigoid as well as for neoplasia such as epitheliotrophic T-cell lymphoma.

**Mucocutaneous pyoderma**
*Clinical signs.* Mucocutaneous pyoderma affects the lips and perioral skin, but also occasionally may affect the nostrils. It occurs more commonly in German Shepherd Dogs, but can occur in any breed. Initial swelling and erythema of the tender lips is followed by crusting and later erosions and fissures. Depigmentation of the lips may also occur.

*Diagnosis.* Diagnosis is made by clinical examination and cytologic evaluation from an impression smear. It is very useful to “peel off” the crust and obtain an impression smear of the underlying erosion. Staining with Diffquick is sufficient — inflammatory cells with intracellular organisms are diagnostic of infection. Biopsy will reveal a band-like infiltrate of predominantly
plasma cells and the disease may be misdiagnosed as discoid lupus erythematosus, but dyskeratotic keratinocytes are not present.

_Treatment_. Antibiotics are most commonly used and will resolve the condition. 2% mupirocin ointment twice daily may also be effective. Unfortunately, many of these patients will relapse and require long term maintenance therapy. The author has identified underlying allergies in some patients and treatment of the underlying disease will prevent recurrence of the mucocutaneous pyoderma.

**Pemphigus**

The pemphigus complex includes four subtypes recognized in human medicine, pemphigus vulgaris, vegetans, erythematosus and foliaceus. In all of these subtypes, the immune system for various and often unknown reasons starts to produce antibodies against parts of the desmosomes, the little connections holding adjacent keratinocytes together. These (auto-) antibodies are called pemphigus antibodies. In the case of the rare pemphigus vulgaris and pemphigus vegetans, the blisters are located suprabasally (which means that the basal keratinocytes are still attached to the basement membrane but the rest of the epidermis lifts off). These deep vesicles rapidly develop into ulcers once burst.

_Clinical signs_. Depigmentation of the planum nasale and/or crusty lesions of the dorsal muzzle, periocular areas and pinnae are often the first signs. Feet, foot pads and groin are affected commonly. Generalization may occur after weeks to months. Pruritus, pain and lethargy may be seen in some cases. Paronychia (with often a caseous, brownish material accumulating in the nail fold) is frequent in cats. One of the classical clinical clues for pemphigus foliaceus is crusting on the inside and nonhaired center of the pinnae. Pemphigus erythematosus is clinically indistinguishable from early pemphigus foliaceus with facial involvement only. Lesions of pemphigus vulgaris are also seen in the oral cavity (80% of the cases have oral ulcers or vesicles at the time of diagnosis), other mucous membranes and the groin and axillae. German shepherd dogs may have lesions on ears and nose only. Lymphadenopathy, anorexia, lethargy and fever as well as secondary pyoderma (and sepsis) are frequently present. These pets are sick, look disgusting and feel miserable. Age, sex and breed predispositions are not known.

_Diagnosis_. Biopsy is the diagnostic test of choice. Multiple biopsies should be taken (four to five samples) and sites should be chosen carefully. Ideally, biopsy intact pustules. Give the pathologist your differential diagnosis list. Suprabasal pustules are filled with neutrophils, acantholytic cells and occasionally eosinophils. The dermis underneath is characterized by a mild to moderate superficial perivascular dermatitis.

_Treatment_. Aggressive immunosuppression is the treatment of choice; the prognosis is poor even with aggressive therapy. Be confident of your diagnosis before beginning immunosuppressive therapy. It can be very dangerous to start immunosuppressive drugs based on history and clinical examination alone. If the animal has an infectious disease (fungal, bacterial or parasitic), it can rapidly deteriorate. There is no place for trial therapy in immune-mediated disease.

The second pitfall is a possible secondary infection. Patients with pemphigus foliaceus often have concurrent bacterial or fungal infection. These patients need to be recognized and treated
for both conditions. In patients with mild to moderate disease, antimicrobial therapy can be started three weeks before immunosuppressive therapy to evaluate how many of the clinical signs are due to the infection and how many are due to the autoimmune disease. With severe clinical disease, treatment of infection and of the autoimmune disease should be commenced concurrently.

Another problem with immunosuppressive therapy is the fact that is impossible to give a good general purpose recipe. Every dog or cat reacts differently to each of the drugs mentioned below. The following recommendations will be a general idea about side effects, mechanism of action and starting doses; these must be individualized for each patient. Immunosuppression is an art requiring instinct, sensitivity and experience as well as the theoretical knowledge.

**Discoid and mucocutaneous lupus erythematosus (DLE)**
Classical DLE is a disease localized to the face and most commonly to the nose (philtrum and planum nasale) of dogs and cats. The exact pathogenesis of DLE is not elucidated in dogs and cats. However, a subset of dogs with clinical signs definitely deteriorates when exposed to UV-light and improves on UV protection. It is safe to assume that UV-rays play a role in at least some of the patients with DLE. Another subset of animals does not seem to be influenced at all by changes in UV exposure. Breed predispositions exist for collies, Shetland sheepdogs, German shorthaired pointers, Siberian huskies and Brittany spaniels. Thus, genetic factors seem to play a role in the disease.

*Clinical signs.* Lupus erythematosus in early stages is characterized by depigmentation, erythema and scaling. Erosions, ulceration and crusting are changes occurring later. Scarring may occur in severe and chronic cases.

*Diagnosis.* Biopsy of skin lesions is the diagnostic procedure of choice. Choose nonulcerated lesions, because the site of action, the dermo-epidermal junction, and epidermis will be obliterated if an ulcer is chosen. A typical finding in formalin-fixed samples is interface dermatitis (inflammation at the dermo-epidermal junction).

*Treatment.* Vitamin E may be administered at 400 to 800 IU daily as safe adjunctive therapy. Scott and Miller reported essential fatty acid supplementation with omega-3/omega-6 fatty acids as an alternative to vitamin E. Concurrently, a combination of doxycycline at 10 mg/kg once daily and niacinamide (vitamin B₃) at 250 (patient <15kg) or 500mg (patient >15kg) every 8 hours for 6 to 8 weeks. If there is no improvement or in severely affected cases, classical immunosuppression is an option.

**Bullous pemphigoid**
Bullous pemphigoid is characterized by autoantibody formation to an antigen in the hemidesmosomes of the basal keratinocytes and subepidermal vesicle formation. Binding of this antibody leads to complement fixation and chemotraction of inflammatory cells, which release proteolytic enzymes resulting in vesicle formation.

*Clinical signs.* Lesions such as depigmentation, vesicles, erosions, ulcers and crusts may occur in the oral cavity, the mucocutaneous junctions and nose.
Diagnosis. Biopsy is the diagnostic tool of choice: subepidermal cleft and vesicle formation with a mild to marked infiltrate of neutrophils and lymphocytes is seen.

Therapy. The prognosis varies with the extent of the lesions. Early mild disease characterized by depigmentation may be treated with vitamin E and/or fatty acid supplementation as well as UV protection. Topical glucocorticoids such as hydrocortisone aceponate, topical calcineurin inhibitors and oral pentoxifylline are drugs useful in disease of moderate severity. Classical immunosuppression may be needed in severe disease.

Erythema multiforme
This is a rare disease that is triggered by drug therapy, viral infections and neoplastic diseases. However, an underlying cause cannot be identified in a number of patients. It is thought to be a host-specific cell-mediated hypersensitivity toward various antigens.

Clinical signs. In small animals, an acute onset of erythematous macules or papules spread peripherally and clear centrally, producing annular or arciform patterns, urticarial plaques and/or vesicles and bullae. Lesions may erode or ulcerate and mucous membranes may become affected. This is usually associated with fever, depression or anorexia. Lesions may also become painful.

Diagnosis. Skin biopsy reveals edema of the superficial dermis, an interface dermatitis and numerous dyskeratotic keratinocytes throughout all layers of the epidermis with satellitosis of lymphocytes and macrophages. Urticarial lesions may show interface dermatitis and severe dermal edema.

Treatment. Mild forms of erythema multiforme may resolve spontaneously. If the disease is more severe, glucocorticoids and/or azathioprin in large doses may be useful. A search for the underlying cause is important. Cyclosporine, etretinate and pentoxifylline have been anecdotally used successfully as well in some patients.

Metabolic epidermal necrosis (MEN)
MEN is a metabolic disorder of unclear pathogenesis and many names. Amino acid deficiency, glucagon excess, vitamin or zinc deficiency in the skin have all been implicated. Underlying reported causes include liver disease ("hepatocutaneous syndrome"), diabetes mellitus ("diabetic dermatopathy"), hyperadrenocorticism, pancreatic glucagonoma ("necrolytic migratory erythema"), which is the term used in human medicine for a disease with similar histopathology caused in the majority of patients by a glucagonoma.

Clinical signs. Severe crusting and erythema is seen predominantly at mucocutaneous junctions (lips, perivulvar/prepuce, perianal area), elbows and hocks and foot pads. Secondary infections with yeast and/or bacteria are extremely common. Dogs with MEN are typically old and sick!

Diagnosis. Biopsy is diagnostic and reveals epidermal hyperplasia and oedema, parakeratotic hyperkeratosis and surface crusting. Since liver disease is often present, serum biochemistry
profiles, liver ultrasound, function tests and/or biopsies are important to determine the underlying disease process and its severity.

*Treatment.* The diet should contain high quality proteins (e.g., added eggs). Vitamin supplementation is useful. In severely affected patients, amino acid infusions are given weekly until improvement is seen (500 ml of amino acids are administered very slowly over 8-10 hours intravenously). Secondary infections are extremely common and control of secondary infections is extremely important for the well-being of these patients. The prognosis for patients with MEN generally is poor, although some rare cases stay in remission for months or several years with appropriate treatment.

**Epitheliotrophic T-cell lymphoma**

“Mycosis fungoides” is a special variety of cutaneous lymphoma, a T-cell tumor characterized by the presence of atypical lymphocytes, which have an affinity for the epidermis and may cluster within epidermal microvesicles (Pautrier's microabscesses). Without the actual determination of the T-cell origin, the tumor is more correctly termed epitheliotropic lymphoma.

**Clinical signs.** The clinical appearance varies tremendously ranging from non-erythematous alopecia with subtle scale, to alopecic plaques, erythematous and/or ulcerated plaques or nodules in the end stage.

**Diagnosis.** When a range of lesions exists on the same animal, the most advanced lesions will be most likely to yield a histopathologic diagnosis. Biopsy of early lesions may reveal only a non-specific inflammatory changes.

**Treatment.** This is a slow growing tumor which is clinically refractory to treatment. The mean survival time following diagnosis is 4 to 7 months. However, earlier lesions of the disease may have been present for years unnoticed. Animals with solitary lesions may sometimes be cured by surgical excision. Treatment is almost uniformly unrewarding. In the author’s experience, the goal of treatment is to give the patient the best possible quality of life, while the disease remains relatively localized. The best clinical response is achieved with glucocorticoids in combination with retinoids.

**Canine eosinophilic furunculosis**

Canine eosinophilic furunculosis is characterized by a sudden onset of a severely pruritic to painful disease on the dorsal muzzle of young, large-breed dogs with access to outdoors. In some cases, exposure to insects such as bees, wasps or fire ants is reported. Although some dogs show clinical signs in winter, most are affected in warmer seasons and arthropod or insect bites/stings are assumed to play a role in the pathogenesis. Recurrences are rare, suggesting that dogs learn to avoid the offending cause.

**Clinical signs.** Papules and nodules rapidly develop into crusts and exudative lesions on the nose. The inguinal area or periocular areas are affected in some cases.

**Diagnosis.** The main differential diagnosis of the acute lesions is a bacterial folliculitis that can be differentiated from eosinophilic furunculosis by cytology. The latter is characterized by
numerous eosinophils; the former by neutrophils with intracellular cocci. Biopsy reveals eosinophilic folliculitis or furunculosis.

_Treatment_. The clinical signs typically respond excellently to glucocorticoid therapy. Prednisolone at 1-2 mg/kg/day is administered until significant improvement is achieved (usually within 1-2 weeks) then tapered for another couple of weeks.

_Zinc-responsive dermatitis_  
**Pathogenesis.** Zinc is an important cofactor and part of many enzymes. Thus, it is involved in many processes in the body. Primary nutritional zinc deficiency is rare in small animals. However, zinc deficiency due to presumed absorption problems is a more commonly encountered entity. It occurs in either young puppies of rapidly growing breeds or in sled dogs such as Siberian huskies or Alaskan malamutes. Bull terriers can also be affected. Breed predilections suggest a genetic influence of the second syndrome. In malamutes, a genetic defect leading to decreased zinc absorption in the intestines has been reported. Detailed studies in other breeds are lacking. The former syndrome is seen in young puppies fed diets high in phytates or calcium (both of which interfere with zinc metabolism). High iron content in drinking water can also inhibit zinc absorption. In both syndromes, the diet contains sufficient zinc but the body cannot utilise it.

_Clinical signs._ Skin lesions in the arctic breeds or bull terriers occur in early adulthood and progress variably. Many dogs are pruritic. Lesions include erythema in the early phase followed by hyperkeratosis, crusting, alopecia around the mucocutaneous junctions (eyes, mouth, prepuce, vulva), chin, ears and foot pads. Thick crusts may also cover the pressure points. Secondary bacterial or yeast infections may develop. Claw disease such as onychomalacia (softening of the claws) may occasionally occur. In rapidly growing breeds such as Great Danes, Doberman pinschers, Labrador retrievers, German shepherd dogs and Rhodesian ridgebacks, hyperkeratotic plaques develop over areas of repeated trauma or pressure points. Foot pads and planum nasale may become hyperkeratotic and deep fissures can develop in severe cases. Secondary infections are not unusual and may lead to lymphadenopathy.

_Diagnosis._ The disease is suspected when the above mentioned breeds present at the appropriate age with the clinical signs compatible of the disease, mainly hyperkeratosis and crusting. Marked parakeratotic hyperkeratosis with eosinophilic serum lakes on histopathology of skin biopsies is compatible with, but not diagnostic of the disease. Cytology will give clues as to the secondary infections.

_Treatment._ With rapidly growing breeds, diet adjustment may resolve clinical signs without any additional treatment but zinc supplementation will enhance and speed up the response. With genetic zinc absorption problems, zinc supplementation is essential. Although the equivalent of 1 mg/kg/day of elemental zinc in form of zinc gluconate, zinc sulphate or zinc methionine is reported as an effective dose, the range in individual dogs goes much higher and varies not only from patient to patient, but also from supplement to supplement. Some dogs will respond to zinc gluconate, but not to zinc sulphate, other dogs vice versa. If a limited response is seen after four weeks, and differential diagnoses have been ruled out, then the dose may be increased. Adding small doses of oral prednisolone (0.5 mg/kg/day or less) have been reported to be useful in some
dogs (possibly due to enhancing absorption, possibly due to decreasing intestinal inflammation due to a subclinical adverse food reaction that is anecdotally suspected in some of these dogs). Concurrent fatty acid supplementation is useful in some patients. In some treatment-resistant cases, intravenous zinc administration weekly at 10 mg/kg of zinc sulphate has been successful in resolving clinical signs, monthly maintenance injections may be needed. Cardiac arrhythmias may be seen if the zinc is administered IV too rapidly.

**Nasal parakeratosis**
Nasal parakeratosis can be seen in young Labrador retrievers. The disease begins with depigmentation but later is characterized by crusting and hyperkeratosis of the planum nasale. A gene test for the responsible mutation in the SUV39H2 gene is available and part of the Official UK Kennel Club DNA Testing Scheme. Topical vitamin E, propylene glycol and petroleum jelly are the treatments leading to partial response in some of those dogs.

**Demodicosis and Dermatophytosis**
Both of those diseases may also cause alopecia, papules, pustules and crusting of the muzzle but are discussed elsewhere in more detail.

**Selected References**
PATHOGENESIS OF OTITIS EXTERNA

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Introduction
Otitis externa (OE) is defined slightly differently in a variety of medical dictionaries, especially when searching online. Most refer to inflammation in the external ear or external auditory canal though some include infection in the definition. Inflammation of the external ear canal is used in this review. OE is a common presenting problem to veterinarians, often being the most common, generally at ≥10% cases. Numerous dermatologic diseases can cause inflammation, which may be sub clinical, in the external ear canal but by the time owners present cases, especially once a case becomes chronic or recurrent, it is a multifactorial problem. The factors can be divided into primary and secondary causes and predisposing and perpetuating factors. Causes are diseases or agents that induce inflammation. Primary causes affect a normal ear and secondary causes occur in an ear that already has otitis externa, generally making the pathology much worse. In this scheme, factors do not directly induce the inflammation but either predispose to primary or secondary causes or occur as a result of inflammation and then promote otitis externa. Perpetuating factors combine with causes or facilitate causes to create more severe inflammation or signs, which then further alters the normal anatomy and physiology of the epithelial, dermal and adnexal structures of the external ear canal.

Normal ears are colonized by bacteria and yeasts and there is evidence that animals with increased cerumen or lipids in their ears may promote or at least be associated with an even greater number of bacteria and yeast. Though some normal ears will not grow any organisms with routine microbiologic culture techniques, microbiome testing has revealed that normal ears harbor numerous bacteria and yeast. Normal ears have greater diversity than otitis externa ears and the most abundant organisms are not the same. One study by Ngo et al evaluated atopic dogs that did not have otitis externa as defined in the exclusion criteria and found that the unaffected ears also have lower diversity and increased abundance of Staphylococcus species and Ralstonia species compared to healthy control ears. It is important to note that the ears could have an OTIS3 score of 4 or less so they were not clinically normal ears. What is not definitively proven, but may occur, is that microbial dysbiosis occurs in an abnormal but noninflamed ears and eventually, without another primary cause, lead to infected ears with inflammation. If this occurs, then infections may occur as a primary or secondary cause.

Primary causes
The parasites Otodectes cynotis (ear mites) and the larval and nymphal stages of Otobius megnini (spinous ear tick) target the external ear but are uncommon in areas where modern parasiticides, especially isoxazolines, are used. Grass awns or other foreign bodies are also significant causes of acute otitis, especially when unilateral. What has not been adequately studied is if they are more common in dogs with ceruminous ears or exudative otitis. Most studies have shown that primary causes are present though one reported 32% of the cases did not have the primary cause determined. Allergy is the most common cause of OE in the dog; two studies, one with 149 dogs and the other 200 dogs, found allergy as the primary cause in over 75% of the cases. An older study of atopic dermatitis indicated that 5% of those dogs had nothing but otitis externa. Atopic otitis can result in signs of aural pruritus even when no visible inflammation is identified. How
often this reflects differences in grading, intermittent presence of visible inflammation or a response to microbial dysbiosis has not been studied, but likely all three play a role. These changes occur initially in some cases with no visible exudate or presence of abnormal cerumen production. Both occur commonly in allergic otitis over time, with the development of various secondary causes, most notably infections. Chronic inflammation in atopic skin leads to epidermal acanthosis and dermal inflammation leads to infiltrates, edema or fibrosis and increased dermal thickness. When these changes occur, they can impact the cartilaginous lined tube in the specific anatomic sites. As the skin thickens it will most likely take up space in the lumen of the ear canal resulting in varying degrees of stenosis. At this point a perpetuating factor, altered epithelial migration, is most often present. Studies proving this are lacking and the exact pathogenesis of how this occurs is unknown. These changes along with failure of epithelial migration will lead to more exudate and debris filling the ear canal. All this exudate and inflammation from the secondary causes and possible aggravated allergic responses and epidermal damage promotes even more perpetuating factors, which eventually become the main problems leading to more severe otitis externa.

**Perpetuating factors**

Inflammation changes adnexal structures as well and histopathologic studies on otitis externa cases reveal varying degrees of fibrosis, sebaceous gland hyperplasia, ceruminous gland (apocrine gland) secretion filled ectasia and hidradenitis. Folliculitis and follicular hyperkeratosis may also be seen. The thickened tissue often results in keratinous filled epithelial folds, which may occlude glandular and follicular ostia. How much these changes are self-promoting or what other causes there are for these changes is unknown. As the proliferation occurs in the lumen of the canal and failure of epithelial migration occurs, the keratinous epithelium builds up within the ear canal. This can exit the orifice of the ear but also may push against the tympanum resulting in dilation of the tympanum and rupture. This leads to otitis media and even possible pseudo “cholesteatoma” formation; in the canine form, the abnormality would not be initiated in the middle ear epithelium. Once perpetuating factors occur and become severe enough, otitis externa continues or recurs even if the causes are resolved. These perpetuating factors also promote resistant or recurrent infections and the keratin can become a substrate for the development of biofilm. Though a study in human beings showed keratinocytes in cholesteatoma can be a substrate for biofilm formation, this has not been studied in canine “cholesteatoma” or keratin within external ear canal folds.

**End-stage otitis**

End-stage otitis is a term used when chronic ear disease results in perpetuating factors that cause severe proliferative tissue and ear canal stenosis, usually associated with otitis media and sometimes associated with calcification. These changes make otitis externa irreversible. End-stage otitis is only successfully resolved with a total ear canal ablation and usually bulla osteotomy. A key to preventing end-stage otitis is the recognition that the different causes and factor that occur in recurrent then chronic otitis externa have different prognosis and treatments. The differences in the pathogenesis of the causes and factors often lead to premature termination of treatments as they are mainly designed to manage the microbial infections and stop the inflammation, but that does not resolve the pathophysiology of the perpetuating factors.
PSPP System was developed to take into account that many of the causes and factors have different prognosis and treatments. Understanding the pathogenesis of how each part contributes to the development of otitis externa is essential, as is realizing their prognosis are not the same.

Selected References
Introduction
Otitis is one of the most common conditions encountered in general practice. Otitis is inflammation of the ear canal. Otitis may be restricted to the vertical and horizontal ear canals (otitis externa) or it may affect the middle ear cavity (otitis media). This usually occurs when infection spreads through the tympanic membrane into the tympanic bullae. Otitis interna refers to inflammation that has reached the cochlea or semicircular canals.

History and clinical signs
The usual clinical signs of otitis are pruritus, pain, head shaking, inflammation of the ear canal, malodor and the presence of a visible discharge. The clinical signs of otitis media are identical to those of otitis externa but they tend to be more persistent and recurrent, and can lead to facial paralysis. Although otitis interna is rare, clinical signs include deafness and vestibular disease (head tilt, nystagmus, ataxia). Further questioning of the owner and a full dermatological examination may reveal signs of a potential underlying cause for otitis. For example, acute onset head shaking is most consistent with a foreign body, whereas a more gradual onset of aural pruritus would be typical of allergic skin disease.

General diagnostic approach to otitis
As with other dermatological problems, a full history and physical examination is required when investigating cases of otitis. The main aim of this is to establish if the dog has any underlying factors that may be causing the otitis (e.g., predisposing factors or primary causes). In particular, dogs should be examined to see if they have any signs of more generalized skin disease. For example, pruritus/erythema/dermatitis of the medial pinnae, face, ventral chest, axillae, ventral abdomen, paws and/or perineum would be suggestive of underlying allergic skin disease. Disease that is strictly limited to one ear canal is more likely to be seen with conditions such as grass seeds, tumours and stenosis. For more details on the pathogenesis of otitis externa, refer to other notes.

In addition, two specific diagnostic procedures should be performed whenever an ear infection is suspected: otoscopic examination and cytological examination of the discharge.

Otoscopic examination
The aim of the otoscopic examination is to:
- Detect foreign bodies or ear mites
- Assess the condition of the vertical and horizontal canals
- Check the appearance and integrity of the tympanic membrane (if possible)
- Characterize the type of exudate that is present.

If the condition is unilateral, clinicians should always examine the good ear first. This prevents the spread of infection from one ear to the other and leaves the most uncomfortable procedure until last. In some cases, the ear canal may be too painful, swollen or full of exudate to allow a meaningful otoscopic examination to be performed. In these cases, the animal can either be
sedated or anesthetised to allow examination, or a preliminary course of treatment can be given and the ear re-examined a few days later. Which course is taken will depend on the severity of the clinical presentation and clinician’s index of suspicion for the various underlying causes. In either case, it is essential that a full otoscopic examination be performed at some stage.

**Cytological examination**
Cytological analysis of the exudate should be performed on the first and all subsequent visits. This can always be performed, even if the ear is too painful to allow a full otoscopic examination. Cytology allows immediate differentiation of the types of infectious agents that may be present (cocci, rods or *Malassezia*).

- *Malassezia* typically occur in the absence of inflammatory cells and are simply present in excessive numbers (over 1 per high power field)
- Cocci typically induce a neutrophilic inflammatory response, but they can occasionally be present on their own
- *Pseudomonas* and *Proteus* would normally induce a severe neutrophilic inflammatory response
- *Corynebacteria* can be present alongside other bacteria or as a pure population. Although they are rods, they are less pathogenic than *Pseudomonas* or *Proteus* and are easily treated with topical agents.

**Culture and sensitivity testing**
Culture and sensitivity testing is not usually indicated if *Malassezia* or cocci are seen on cytology because the sensitivity profile of these organisms can be predicted with reasonable certainty. The presence of rods should prompt the clinician into performing bacterial culture and sensitivity testing because resistance is much more of a problem with gram negative organisms. However, bear in mind that if the rods are *Corynebacteria*, they may not be reported by the laboratory as they are not considered highly pathogenic. Cultures should also be performed on cases of bacterial infection in which the cytological picture has not changed after a course of treatment, because this may indicate bacterial resistance.

**Diagnosing the underlying cause**
In addition to diagnosing and treating the infection itself, the clinician must also try to determine the underlying cause. This is especially important in any case that has suffered otitis on more than one occasion. If this is not done, many cases will become recurrent or persistent. In addition to history, physical examination, otoscopic examination and cytology, the diagnosis of the underlying cause may require:

- Investigation of suspected allergic skin disease
- Video-otoscopic examination to allow detailed examination of the ear canals and tympanic membrane
- CT or MRI of the ear canals and tympanic bullae
MANAGEMENT OF ACUTE OTITIS EXTERNA
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Introduction
Acute otitis externa is primarily caused by ear mites, foreign bodies and atopic disease with or without secondary infections. Atopic otitis is often how atopic dermatitis begins and these cases will typically be recurrent, though initially may have months between presentations. It is also important to recognize the earliest signs of disease in these cases and to intervene as soon as possible so that effective perpetuating factors are prevented. A study assessing pruritus signs in 314 healthy dogs also assessed head shaking and ear cleaning. Head shaking was reported as never noticed by 33% of owners and only 5% noted it occurring multiple times a day while 11% noted it daily. This often is the first sign seen by owners of dogs with acute otitis and once owners are educated about acute otitis, they often notice an odor or other pet licking the affected dogs’ ears as the earliest sign a problem is occurring. The owners of the healthy dogs were asked about ear cleaning and (146) 47% did indicate that they cleaned their dogs’ ears. Their veterinarian had told 7% of those owners to clean their dogs’ ears but we do not know why that was done. More interesting, 27% indicated if they did not clean the ears, the ears got dirty, filled with debris and/or smelled. This could be occurring because even in dogs who had reportedly never had any skin or ear problems and never needed a medicated ear treatment, they had some ear situation that the owners had learned could be controlled with ear cleaning.

Cleaning the ears
Assessing the acute otitis externa case should include a thorough otoscopic examination to determine if there is bilateral or unilateral disease and if there are ear mites, gross foreign bodies, alterations in epithelial lining of the ear canal or tympanic membrane, or excessive cerumen or exudate within the ear canal. It is also important to take a good history and determine if this is a recurrent case of acute otitis and if there are any signs that suggest the pet has allergic disease. Cytology is performed from the ear canals to determine microbial infection or overgrowth and to determine if it is yeast, coccii (most likely staphylococci) or rods (most often Klebsiella, Escherichia, Proteus and Pseudomonas organisms). One study in a general practice setting in Nova Scotia, Canada where most cases were not chronic found only 8% of otitis externa cases had Pseudomonas organisms.

Ear cleaning is indicated if foreign bodies are identified, excessive cerumen or exudate are present, the case has a recent recurrent acute episode or allergic disease is present. In some cases, especially those that are allergic or recently recurrent and that do not have excessive cerumen or exudate, ear cleaning may be done by the clients at home. If there is so much cerumen or exudate that the tympanum is not visible, then in-office cleaning is preferred so the state of the tympanum can be determined. When foreign bodies are identified, in-house cleaning is also indicated after the foreign body is removed. There may be residual small or even foreign microscopic material present that require a good cleaning. In these cases it is best not to rely on the owner for effective home cleaning.

Home ear cleaning may be the only treatment needed in uncomplicated atopic otitis cases. When cytology reveals only yeast or bacterial overgrowth (and even in some cases of secondary
infection), cleaning by itself may be sufficient to resolve the otitis and can be used to prevent further recurrences. Intermittent home ear cleaning is often all that is needed in atopic otitis cases to prevent recurrences and progression to perpetuating factors; some cases may need intermittent topical glucocorticoid therapy as well. When infection is present, it may be indicated to use an antiseptic rinse in addition to the ear cleaning protocol. There are several methods of home ear cleaning though for most cases just a thorough flushing technique is satisfactory. In this case, an ear cleanser with some antimicrobial activity is selected. The key to success is making sure clients have been instructed in the proper technique. When I ask clients how they clean, they commonly describe a technique which I find less than satisfactory for most cases. Often, they use little flushing fluid and concentrate on physical wiping with some material or device to remove material from the external orifice. This commonly fails to effectively clean a dogs ear, especially the horizontal canal.

Proper flush techniques are rarely taught in the manner that the author prefers. The “Clean Until Clean Cleanser” approach is preferred. My clients are taught a thorough flushing technique that involves filling the ear with the cleanser and not applying a set number of drops. The ear is filled with fluid until the fluid is seen at the orifice. The ear canal is then gently massaged to help break up debris and develop fluid waves within the ear canal. Clients should be instructed how to gently squeeze or push against the cartilage of the vertical canal but then to also gently move their finger under the horizontal canal and push up to also move the fluid and help break up material in the horizontal ear canal. While this is occurring, much of the fluid will flow out of the external orifice, which means the cleaning fluid can no longer be seen in orifice of the ear canal. At this point (or if the dog has shaken its head and removed all the fluid), more cleaning fluid is added to fill the ear again. This is an important part of the ear cleaning technique; it is imperative the client tries to, at least briefly, prevent any head shake, massage or dropping of the ear. The client should observe the cleaning fluid and determine if it is cloudy or if flecks of debris are still present. If so, gentle massage is again performed. This process is repeated up to three times as too much cleaning with massage can be irritating. Clients generally use this cleaning technique once weekly. When done well, this is generally all that is needed to keep an ear clean for at least one to two weeks, especially in less complicated cases with no or minimal perpetuating factors. If the next cleaning reveals that the cleaning fluid is relatively clear (similar to how it looks straight out of the bottle), then the ears are most likely cleaned well. This is also checked by scheduling follow ups so that the client cleans the ears the morning of their appointment or the night before.

Topical glucocorticoids

Acute otitis cases that have visible inflammation are usually treated with topical glucocorticoids, though this is not always necessary with acute otitis induced by ear mites. In some atopic otitis cases, the topical glucocorticoids are needed to control the pruritus and inflammation as cleaning alone may not be effective. In most atopic otitis cases, the concave pinna near and into the external orifice is affected and the topical glucocorticoids should be applied in this area not just the ear canal. If inflammation is seen extending up the concave pinna then those areas are also treated. Typically, treatment is initiated daily until resolution of signs and at least 3 to 5 more days. If the acute otitis has been recurrent or if cleaning alone is ineffective, then this treatment may be needed after each ear cleaning to prevent future flare ups. One 16-week maintenance study by Bergvall et al 2017 evaluated topical hydrocortisone aceponate given two days weekly,
without cleaning, to prevent recurrences of acute otitis in allergic dogs. At the end of 16 weeks, 50% of the placebo had flared, while only 18% with treatment. For initial therapy of acute otitis, a more potent glucocorticoid is most often initially used. These would include 0.1% mometasone furoate, 0.11% hydrocortisone aceponate, 0.1% dexamethasone, and 0.1% betamethasone. Though it may be a little less potent, prednisolone acetate 0.5% is also used by the author when combined with other ingredients (miconazole 2.3% and polymyxin B sulfate 0.053%, Surolan [Elanco]).

**Topical antimicrobials**

Topical antimicrobial drugs include antiseptics, antibiotics and antifungal agents. In most acute otitis cases, a topical combination product will be used that contains an antibiotic and glucocorticoid, and possibly an antifungal agent. First line products for cases that have never had otitis and topical antibiotic therapy are often treated with neomycin or polymyxin containing products. These two antibiotics are generally effective for most staphylococcal or mixed infection cases and less likely to promote resistance to commonly used antibiotic classes. There are now two long acting combination products that can be applied in clinic after the cleaning. These are Osurnia (Elanco) and Claro (Bayer). Both contain florfenicol as the antibiotic and terbinafine as the anti-yeast agent, though the concentrations differ. Osurnia contains betamethasone and Claro contains mometasone furoate 0.22%, the highest concentration available in a veterinary product. In published studies, Claro seemed a little more effective (73%) than Osurnia (65%), but the studies used different scoring systems and, more importantly, different criteria for success.

If one is trying to avoid using topical antibiotics, there are a variety of antiseptic containing products available, but they do not typically contain a glucocorticoid. Depending on the etiology of acute otitis, these may be effective when combined with foreign body removal, effective systemic treatment for ear mites (isoxazoline) and through cleaning. A short course of oral prednisone 1 to 1.5mg/kg or short acting glucocorticoid injection may also be indicated. If a dog has allergic otitis, then cleaning and antiseptic drops combined with a Cytopoint injection or Apoquel therapy may be helpful. In countries where compounding is permitted, anecdotal success has been reported when combining injectable dexamethasone phosphate with the antiseptic at concentrations ranging from 0.1 to 1mg/ml. The higher concentration is the more typical one found in prescription veterinary ear products with dexamethasone.

**Selected References**

Management of Chronic Ear Infections

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Chronic ear infections can be defined in a couple of different ways—both clients and clinicians often confuse the two. Chronic ear infections may refer to:

- Recurrent ear infections – this means that the infection responds to topical treatment when it is given, but the condition relapses sometime after the treatment is discontinued. Often this leads to multiple bouts of otitis being treated over a period of time.
- Persistent or refractory ear infections – this means that the otitis does not respond to treatment when it is given. The infection would still be present when the animal was rechecked at the end of a course of treatment (discharge still present, infection is still evident on cytology).

When presented with either of these scenarios, the answer always lies in the presence of an underlying factor or perpetuating factor that has not been adequately diagnosed or treated. It is tempting for clinicians to simply try another ear drop to see if that will work, but this rarely results in a satisfactory outcome.

The diagram on the following page illustrates the various underlying causes of otitis in a novel way. This diagram combines the various predisposing factors and primary causes into a single category of initiating causes, but adds in the likelihood of that particular problem actually leading to clinically relevant otitis. The chart does not indicate how common the various conditions are. They are ranked in order of how likely the various conditions are to result in otitis.

The chart also shows the perpetuating factors that result from either microbial evolution, or progressive pathology in the ear canal.

The initial approach in any chronic ear infection is to try and identify which of these various conditions or pathologies is present in the patient. Treatment of the ear infection itself is not likely to be successful if one of these conditions is underlying the problem.

The following notes provide guidelines on treating these various problems.
Pathophysiology of Otitis and Ear Infections

Initiation of otitis
Possible causes and risk of otitis developing

- Grass seed foreign body
- Ear canal polyps and tumours
- *Otodectes cynotis* infestation (ear mites)
- Atopic dermatitis
- Food allergy
- Abnormal or disrupted natural ear cleaning mechanism
- Adverse reaction to ear medication
- Endocrine disease
- Transient changes to ecosystem
- Otic demodicosis
- Swimming
- Congenitally narrow ear canals
- Hairy ear canals
- Excessive wax production
- Pendulous pinnae

MICROBIOME DYSBIOSIS and EAR INFECTION

Perpetuation of otitis
Microbial evolution and progressive pathology

- Resistant bacterial infections (esp. Pseudomonas)
  - Often follows previous treatment
  - Survival of the fittest in an antibiotic rich environment
- Ruptured tympanic membrane and otitis media
  - Often occurs spontaneously due to digestive enzymes in pus
- Ear canal hyperplasia, fibrosis and narrowing
  - Can inhibit penetration and dispersal of treatment agents
- Development of ceruminoliths
  - Can act as a nidus of infection
- Damaged natural ear cleaning mechanism
  - Will provide a constant environment for microbiome dysbiosis
- Excessive cleaning
  - Can cause irritancy and maceration
Treatment of the Initiating Causes

Grass seed foreign body
Grass seeds need to be identified on otoscopic examination and then removed using crocodile forceps. This may need to be performed under sedation or general anesthesia. The tympanic membrane should be checked for perforation. A course of ear drops can be prescribed to deal with any secondary infection.

Ear canal polyps and tumors
Tumors of the ear canal typically occur in middle age to older dogs and cats. However, polyps can occur in younger cats. These polyps originate in the Eustacian tube and can either grow into the nasopharynx or extend into the middle ear and ear canals. Ear canal tumors need to be surgically resected and this may require a lateral wall resection or total ear canal ablation (see below). Ear canal polyps in cats can be removed by surgical traction (literally pulling them out). A course of prednisolone after removal can help to reduce the risk of recurrence, but this may still occur in about 50% of cases.

Otodectes cynotis infestation (ear mites)
This would be most common in puppies and kittens. Typically, a dry, crumbly brown discharge is seen in the ear canal. Ear mites can usually be visualised on otoscopic examination. Although ear drops may drown the mites, the most appropriate treatment would be a systemic acaricide such as selamectin, moxidectin or an isoxazoline (check species and age restrictions).

Atopic dermatitis
Allergic otitis is very common in atopic dogs. Typically, there would be inflammation of the medial pinnae in addition to the ear canals. Long term management of the atopic dermatitis is essential in order to prevent repeated episodes of otitis and ear infections. In some dogs, the overall management of the atopic dermatitis with oclacitinib (Apoquel), lokivetmab (Cytopoint), ciclosporin (Atopica), prednisolone or immunotherapy will control the otitis. In other dogs, some additional localized treatment is required to prevent recurrences. This usually involves a once or twice weekly application of a topical glucocorticoid such as Elocon (mometasone) lotion, Elocon cream or Cortavance (hydrocortisone aceponate). If Malassezia overgrowth is a recurrent problem, intermittent use of Surolan/Dermotic/Apex PMP may be required.

Food allergy
Food allergies can lead to otitis along with more generalized cutaneous signs. If the condition can be controlled by dietary management, the otitis should not recur. If recurrences do occur, they should be controlled as described above for atopic dermatitis.

Abnormal or disrupted natural ear cleaning mechanism
As this can be both an initiating cause of microbiome dysbiosis, as well as a perpetuating factor in ear infections, it is covered at the end of these notes.

Adverse reaction to ear medication
Some dogs may experience an adverse reaction to an ear medication. This is most common with ear cleaners. Some of the acidic ear cleaners may cause immediate irritation (stinging) if there is ulceration of the ear canal lining. Even without ulceration, some ear cleaners are irritating to
dogs, especially if used excessively. This can cause inflammation and trigger otitis. Excessive use of ear cleaners will also cause maceration (see below). Dogs may also become allergic to certain ear medications. If any of these situations are suspected, an alternative product should be used.

**Endocrine diseases**  
Dogs suffering from hypothyroidism or hyperadrenocorticism can develop otitis and ear infections. The cause is multifactorial and can include changes to cornification or glandular secretions, impaired resistance mechanisms or disruption to the natural ear cleaning mechanism. Diagnosis and treatment is covered elsewhere in the WCVD program.

**Transient changes to the ecosystem**  
Occasionally, transient changes in temperature or humidity may trigger microbiome dysbiosis. This could be due to various factors, some of which may never be identified. The end result could be an infection for which no underlying cause can be found and which resolves after a single course of treatment.

**Otic demodicosis**  
Otitis can be seen in dogs suffering from generalised demodicosis. In this situation, *Demodex* mites can be found within the ear canal and observed microscopically on samples obtained using swabs. Treatment of the skin disease with isoxazolines should resolve the otic demodicosis.

**Swimming**  
Many dogs go swimming without getting otitis, but it can lead to environmental changes within the ear that trigger microbiome dysbiosis. If this is a recurrent theme, treatment of the ears after swimming with a drying ear preparation may help to stop infections developing. An alcohol/acetic acid combination as is used in people may be beneficial.

**Congenitally narrow ear canals**  
Congenital stenosis of the ear canals is seen in some breeds, especially Shar Peis and brachycephalic breeds (bulldogs, French bulldogs, pugs). This does not always lead to otitis, but it may make otitis more likely if a dog had another initiating cause concurrently. The end result would be failure of the natural ear cleaning mechanism. Treatment options include a routine ear cleaning regime or surgical alteration of the ear canals (see below).

**Hairy ear canals**  
Some dogs (especially poodles) have large numbers of hair follicles in the ear canals. In some dogs, this may cause no problems, but in others it can results in disruption to the natural ear cleaning mechanism. If the dog is not having any problems, the ears should be left alone. If the excessive hair is resulting in otitis and infections it may be necessary to have the hair plucked out at regular intervals. This can be done by a groomer, but a short course of glucocorticoid ear drops is advisable afterwards to prevent the development of inflammation. A maintenance ear cleaning regime may also be beneficial, as described above.
**Excessive wax production**
This may be due to physiological variation between dogs and breeds, but it may also occur secondary to some inflammatory disease process. In many dogs, a bit of excess wax may not lead to any problems and the owners may just remark that the dog always has slightly “dirty” ears. However, in other situations, the end result could be a failure of the natural ear cleaning mechanism.

**Pendulous pinnae**
The vast majority of dogs that walk around with pendulous pinnae do not get otitis. It is not, therefore, a common cause of otitis by itself. However, the presence of pendulous pinnae may make otitis more likely if a dog had another initiating cause concurrently.

**Treatment of the Perpetuating Factors**
Perpetuating factors can be the most troublesome to deal with because they can make the ear infection become recurrent or persistent. If perpetuating factors are allowed to gain momentum, the ear canal can become permanently and irreversibly damaged. The best way to deal with perpetuating factors is to stop them developing in the first place by following the diagnostic and therapeutic guidelines outlined above. The treatment of some perpetuating factors requires considerable experience and expertise and can involve the following:

- Treatment of resistant infections
- Management of middle ear disease (otitis media)
- Managing proliferative changes

These treatments can involve complex medical or surgical treatments and are best dealt with by specialist dermatologists and surgeons.

**Resistant bacterial infections, especially *Pseudomonas* otitis**
Some cases of otitis externa develop an infection with *Pseudomonas aeruginosa*. This most commonly follows treatment of a pre-existing ear infection with various anti-microbial agents. This organism can be resistant to many commonly used antibiotics and can be difficult to treat. It also frequently results in otitis media.

The following products may be useful in the management of cases of otitis externa caused by this organism, although antibiotic selection should be guided by culture and sensitivity results.

1. Gentamycin ear drops—do not use if eardrum is ruptured (which it often is).
2. Aurizon ear drops—contain marbofloxacin which may be effective even if a culture and sensitivity indicates resistance. This is because the resistance of the organism is dose-dependent and can be overcome by the large concentrations achieved when the antibiotic is applied topically.
3. Baytril Otic ear drops (contain enrofloxacin and silver sulfadiazine). This combination may still be effective, even if a culture and sensitivity test indicates resistance.
4. Otoflush (TrizEDTA)—this solution potentiates the action of aminoglycosides and possibly fluoroquinolones. It may also be directly bactericidal for *Pseudomonas* organisms. The solution can be used as a flushing or cleaning agent prior to the application of the topical antibiotics mentioned above.
5. Alcohol/acetic acid flushes
6. Compounded antibiotic formulations containing antibiotics such as ceftazidime, aztreonam, tobramycin or piperacillin (these preparations should be prescribed by specialists).

**Treatment of otitis media**
If the tympanic membrane has ruptured, the clinician is faced with the treatment of otitis media. In some cases, this can be managed medically by middle ear flushing followed by aggressive antibacterial therapy. Systemic therapy may be indicated as well as topical, but is not always necessary. Treatment of such cases is best undertaken by a specialist dermatologist using video-otoscopy equipment. In some cases, the damage to the middle ear is permanent and surgical management is required (see surgical techniques below).

**Ear canal hyperplasia, fibrosis and narrowing**
In some cases, this can be reduced or reversed with high doses of glucocorticoids.

**Treatment of abnormal or disrupted natural ear cleaning mechanism**
The natural ear cleaning mechanism prevents a build-up of wax and dead skin cells in the ear canal. It relies on epithelial migration of skin cells, which carry wax and debris out of the ear canal. If this mechanism is abnormal or disrupted, wax and epithelial debris will accumulate in the ear canal. This can be easily observed on cytology. This problem is only commonly recognized in dogs. It is not a problem in cats.

This problem can occur in the following circumstances:
- If the production of wax or skin cells is excessive
- If the ear cleaning mechanism is inhibited by excessive hair
- If the ear canals are stenotic
- With any disease that damages the epithelial lining of the ear canal (including allergies and infections)
- If the problem is not addressed, the excessive wax can form into hardened masses known as ceruminoliths.

Disruption to the natural ear cleaning mechanism means that the ears have to be artificially cleaned. A single thorough flush will not have long lasting effects and is not usually beneficial on its own. An ear cleaning regime will be necessary if the ear cleaning mechanism is permanently disrupted. However, it is critical to avoid over-cleaning, because this can lead to irritancy and maceration, which will cause further problems. *Ear cleaning in cats is rarely indicated and can cause more problems than it solves.*

**The technique and frequency of routine ear cleaning**
If successful results are to be obtained, owners must be given a demonstration of this ear cleaning technique. While holding the pinna firmly, the ear canal is flooded with the ear cleaner. The cartilage of the vertical and horizontal canals is then massaged. Owners must be shown how to achieve deep palpation if the technique is to be effective. The appropriate technique produces a characteristic “squelching” sound. The dog is then allowed to shake its head which removes much of the liquid. Any residual cleaner should then be removed with a gauze swab or cotton pad. Cotton buds should be avoided because they can push debris deeper into the ear canal. Owners should be told to inspect the gauze swab carefully to see what comes out. This is very important when ear cleaning is being used on a long-term basis because it can be used to
determine the frequency of application. If the swab is very dirty, the ears should be cleaned again the next day. If the swab is completely clean, the ears can be cleaned every other day. If the swab is still clean, twice weekly cleaning can be initiated, followed by once weekly if appropriate. Once the swab starts to look dirty again, the correct frequency of application has been determined.

A number of products are available for cleaning dog’s ears. These contain various ceruminolytics (dissolve wax), keratolytics (break down skin cells), antiseptics and drying agents. The choice of ear cleaner is often a case of personal preference, but there are some differences. If an ear cleaner is irritant in some dogs, it should be changed to a gentler option.

**Surgical management of otitis**

Surgery can be used as an adjunctive treatment in the management of otitis, or in some cases, it can be curative. The use of surgical techniques in the management of otitis is summarised below. Clinicians should remember that surgery is unlikely to be helpful in cases of otitis secondary to allergic skin disease because the inflammatory changes will persist in the remaining tissue and the pruritus will not resolve.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Indications</th>
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<tr>
<td>Lateral wall resection</td>
<td>Poor ventilation due to pendulous pinnae</td>
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<td></td>
<td>Poor ventilation due to narrow ear canals</td>
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<td>Poor ventilation due to hairy ear canals</td>
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<td>Otitis as part of seborrhoeic conditions</td>
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<td>Ceruminous gland hyperplasia in the vertical canal</td>
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<td>Neoplasia in the vertical canal</td>
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<td>As an aid to the management of recurrent otitis externa or media</td>
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<tr>
<td>Vertical canal ablation</td>
<td>Fibrosis of the vertical canal</td>
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<td></td>
<td>Proliferative changes on the medial aspect of the vertical canal</td>
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<td></td>
<td>Ceruminous gland hyperplasia in the vertical canal</td>
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<td></td>
<td>Neoplasia in the vertical canal</td>
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<td>Total ear canal ablation and</td>
<td>Persistent or refractory otitis media</td>
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<td>bulla osteotomy</td>
<td>Osteomyelitis of the bulla</td>
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<td>Fibrosis of the horizontal canal</td>
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<td>Calcification of the horizontal canal</td>
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<td>Neoplasia in the horizontal canal or bulla</td>
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Introduction
An intact hair coat requires correct hair follicle morphogenesis during embryonic life and lifelong recurrent hair growth. After initial follicular morphogenesis, the hair coat is preserved by repetitive cycles of the hair follicles through periodic stages of growth (anagen), regression (catagen), and quiescence (telogen). Hair shedding occurs during the exogen phase and follicles that lack a hair shaft are in the kenogen stage (hairless telogen). The duration of the different hair cycle phases varies between dog breeds and body locations and are influenced by age, sex, hormones and day light. This may result in shorter or longer hair on specific body parts, a thicker or thinner winter or summer hair coat and variations between animals of the same breed. Most dog breeds have a mixture of anagen (average 40%), telogen (average 17%) and kenogen (average 14%) hair follicles but some breeds (e.g., the poodle) have an anagen-dominated cycle with more than 90% of the follicles being in anagen. Due to the huge breed dependent variations in hair fiber thickness, hair follicle density and seasonal changes, the clinical and histological assessment of mild alopecia may be difficult and breed specific aspects have to be taken into account.

Alopecia or hypotrichosis can be primary or secondary and, in primary cases, it is either non-inflammatory or inflammatory. Non-inflammatory alopecia is attributed, at large, to a decreased formation or cytodifferentiation (in most cases hereditary) or an impaired regeneration (in most cases acquired, but can have a hereditary background) of hair follicles. Frequent conditions associated with non-inflammatory alopecia include hair cycle disorders of endocrine origin (e.g., hypothyroidism, hyperadrenocorticism, hyperestrogenism), hair cycle disorders of unknown origin (e.g., alopecia X, recurrent flank alopecia), hereditary conditions (e.g., ectodermal dysplasia, follicular dysplasia) and follicular atrophy caused by ischemia (e.g., dermatomyositis, rabies vaccine induced alopecia). Inflammatory alopecia is caused by an increased destruction of hair follicles and/or hair shafts. The cause can be either infectious or immune-mediated. In most cases, inflammation targets primarily the hair follicle; however, the hair follicle can also be affected by inflammatory cells that target other components of the skin (e.g., sebaceous glands). Examples of inflammatory alopecia are folliculitis caused by infectious agents (bacteria, dermatophytes or demodex mites), perifollicular inflammation caused by leishmania or immune-mediated follicular or perifollicular inflammation (e.g. alopecia areata, idiopathic mural folliculitis, perifolliculitis, sebaceous adenitis). Alopecia as a secondary lesion develops subsequent to dermal inflammation and pruritus due to hypersensitivity reactions or ectoparasites.

Alopecia is a common cause for consulting a veterinarian and a systemic work-up is mandatory to identify the correct underlying cause. This allows successful management of the case. The general diagnostic approach for non-inflammatory or inflammatory alopecia is similar. In many cases, the complete signalment, history and the results of a thorough general and dermatological clinical examination allow the decision if the cause of alopecia is inflammatory, non-
inflammatory or secondary. Further specific laboratory tests are then needed for the final diagnosis.

**Signalment**
Information about breed, age, sex and coat color of the animal is important when assessing the cause of alopecia. In dogs, non-inflammatory alopecia at birth is associated with a hereditary cause (follicular dysplasia), whereas in other animals, congenital alopecia may also be the consequence of a metabolic imbalance (e.g., maternal iodine deficiency) or an *in utero* infection (e.g., BVD). In hairless dog breeds (Chinese crested dogs, American hairless Terrier, Peruvian Inca orchid and Mexican hairless dog), the specific gene variant resulting in alopecia has been propagated by breeders. If congenital alopecia is seen in mongrels the breeds or phenotype of the parents should be considered, since matings of hairless and haired dogs may result in hairless puppies. In many forms of hereditary alopecia, dogs are born with a normal hair coat and alopecia manifests within the first few years of age and progresses over time. A clear breed predisposition to develop follicular dysplasia postnatally has been observed worldwide or in certain countries only. Examples are color dilution alopecia in some dilute dog breeds, such as the doberman pinscher or the silver Labrador retriever. Other examples of alopecia with a clear breed predisposition have been described in curly coated retrievers, Chesapeake Bay retrievers, Irish water spaniels, and Portuguese water dogs. Breed predispositions not restricted to a young age are also seen in acquired pattern alopecia in breeds such as Manchester terriers, miniature pinschers and dachshunds. Cyclic recurrent flank alopecia is seen in the doberman pinscher, boxer, airdale terrier, Rhodesian ridgeback and English bulldogs. Alopecia X is seen in Pomeranians but a similar clinical condition has also been reported in other plush coated dogs such as the chow-chow, keeshound or nordic dog breeds. Whether the alopecia in these breeds has the same pathogenesis as in Pomeranians remains to be investigated.

Some forms of inflammatory alopecias also show clear breed predilections and are seen in young dogs. American Staffordshire terrier, Staffordshire bull terrier, Chinese shar pei, French bulldog, English bulldog, pit bull and Sealyham terrier are predisposed to develop generalized juvenile demodicosis and breeds such as akita inu, standard poodle, viszla, hovawart, Havanese and English springer spaniel develop more frequently sebaceous adenitis during adulthood.

**History**
A profound patient history prior to the clinical examination is important in the diagnostic process. Beside the before mentioned information outlined under signalment, the age of onset of the alopecia, the progression, the environment, previous illness, sex and reproductive history, signs of internal disease, stress, response to treatment, type and time of ectoparasite control and clinical signs in other animals or contact persons should be inquired. Whereas non-inflammatory alopecia in young dogs is more likely to be inherited, older dogs rather develop endocrine based forms of alopecia. Many endocrine diseases resulting in alopecia have concurrent signs in other organ systems (e.g., polyuria, polydipsia and polyphagia in hyperadrenocorticism). Dogs with estrogen producing tumours or bitches with ovarian disorders may develop sex hormone imbalance (e.g. hyperestrogenism) resulting in alopecia. Cyclic episodes of non-inflammatory alopecia are suggestive of recurrent flank alopecia. Stress or previous illness may result in telogen effluvium, anagen defluxion or alopecia areata. Doxycyclin therapy in breeds with an anagen dominated hair cycle may cause anagen effluvium. Focal alopecia may be the result of
drug administration and may become diffuse if drug-associated ischemia is the underlying cause. Dermatophytosis is seen more frequently in hunting dogs and dogs housing together or living in warmer environments.

Clinical examination of hair coat and skin
A general clinical examination should precede the dermatological examination to exclude systemic illness as underlying cause for the alopecia (e.g., immunosuppression and subsequent alopecia of infectious cause). The clinical dermatological examination may distinguish between non-inflammatory and inflammatory alopecia.

The distribution (focal, multifocal, symmetrical, or asymmetrical) and the degree of the hypotrichosis/alopecia should be noted. Symmetrical alopecia is usually associated with non-inflammatory alopecia, while non-symmetrical, patchy alopecia is mainly associated with skin inflammation. This distinction is only valid in acute cases; thus, it is essential to ask the owner how lesion distribution was at the beginning of the hair loss.

Clinical signs of secondary pruritus (e.g., broken hair shafts and signs of self trauma) are often associated with inflammatory alopecia. However, it needs to be emphasized that most alopecias, independent of the primary cause, may be eventually associated with secondary bacterial infection and, consequently, signs of inflammation are present. Coat color changes (staining) are often seen with inflammatory alopecia as a consequence of intensive licking and the release of inflammatory mediators in yeast or bacterial infections. Other clinical signs of inflammatory alopecia caused by primary infections are folliculitis, follicular pustules, collarettes, follicular crusts or the presence of infectious agents. If the above mentioned signs of inflammation are not present, signs of non-inflammatory alopecia including hyperpigmentation, or depigmentation of skin or hair coat should be assessed.

A dermatoscope may be helpful to decide if alopecia is inflammatory or not since it allows the close examination of haired skin. Morphologic changes not so easy visible by the naked eye can be recognized. These include subtle and early signs of inflammation, the form and origin of scales, follicular casts, presence of primary and secondary hair shafts, broken hair shafts and hair shaft abnormalities. Hair shafts affected by dermatophytes, demodex mites and flea feces can be detected more easily with a dermatoscope.

Trichography
Trichography is a non-invasive, valuable diagnostic tool to examine hair shaft changes seen in inflammatory and non-inflammatory alopecia. It is best performed by a rubber-covered hemostat, pulling the hair in the direction of hair growth to minimize trauma and mounting the hairs in the same orientation on a microscope slide with mineral oil and covering by a cover slip. Plugged hairs allow the detection of demodex mites and dermatophytes, follicular casts and fragile, misshaped hair shafts. Details for the usefulness of trichograms in non-inflammatory alopecia are lined out below.

Skin biopsy
Skin biopsies are not a first-line diagnostic tool to diagnose inflammatory, non-inflammatory or secondary alopecia. However, they can add valuable information in alopecic diseases, if the
findings resulting from the other mentioned methods are not sufficient to diagnose the underlying cause. Since biopsies may be used as diagnostic aid for all forms of primary and secondary alopecia, basic principles about this technique are presented already here, although other clinical tests should be performed prior to biopsies.

Good biopsy results can only be achieved if the following points are considered:

1. A list of differential diagnoses should be made before taking the biopsy. This allows the correct location for biopsies to be determined and the opportunity to select primary skin lesions. Both are necessary to get the most specific histopathologic result.
2. The biopsy should be taken before treatment, or three to four weeks after treatment withdrawal. Specifically, corticoids alter the hair cycle rapidly and should be discontinued for at least 4 weeks before biopsies are taken.
3. Instruments for biopsies need to be sharp and fine and should be used delicately to avoid artefacts, which if severe, interfere or obscure the histopathological interpretation.
4. Whenever possible skin samples need to be of sufficient size and number to yield enough hair follicles for analysis. Furthermore, biopsies should be taken deep enough to deliver subcutaneous fat since anagen bulbs are located in the subcutis. To diagnose the cause of alopecia, at least 2 to 3 punch biopsies (6 to 8 mm in diameter) from the center of alopecia should be taken. In addition 1 to 2 samples from a recently expanding margin, staying just inside the area of well-developed alopecia should be collected.
5. Active areas within primary skin lesions, usually identified by erythema and recent development, extension, or expansion are biopsied. Avoid biopsy of healed lesions.
6. In subtle, unusual, or non-inflammatory regional alopecia, also biopsy a normally haired area of a similar anatomic location, to allow comparison with alopecic skin.
7. Biopsy normal haired skin in breeds with an unusual hair coat to allow comparison to normal.
8. If follicular dysplasia is suspected, always biopsy, if present, haired skin to allow comparison to normal.
9. Mark the orientation of hair growth on the alopecic skin surface with a line using a permanent marker ink and biopsy with the line in the center of the punch. This allows correct trimming and orientation of the biopsy in the laboratory.
10. Fix the biopsies immediately in 10% buffered formalin and ship the specimens in different containers with a thorough history, description of the lesions, laboratory results, previous treatments and treatment response and a differential diagnosis.
11. Note that biopsies cannot always reliably differentiate the cause of non-inflammatory alopecia, since with chronicity they look more and more alike histologically.

Further assessment of inflammatory alopecia with infectious cause
Demodicosis and dermatophytosis can mimic non-inflammatory alopecia and signs of inflammation can be sparse. Therefore, dermatophytosis and demodicosis should always be ruled out. If inflammation is more pronounced, a bacterial or mycobacterial cause becomes more likely. All infectious causes of inflammatory alopecia can be narrowed down by the use of additional methods.
**Dermatophytosis.** Dermatophytosis is diagnosed by utilizing complementary diagnostic tests, including the collection of hairs, crust, or scale from the periphery of expanding skin lesions (not from the center) for trichoscopic examination and culture. If *Microsporum canis* is present, the use of a Wood's lamp to collect fluorescing hairs from untreated dogs may enhance diagnostic accuracy. Since dermatophytes can be missed in plucked hairs, the Mackenzie brush technique increases the likelihood to collect samples with dermatophytes for culture. Culture allows to identify fungal species involved as does PCR. However, PCR may also be positive if only dead fungal organisms are present in the sample.

**Demodicosis.** In most cases of inflammatory alopecia, deep skin scrapings are warranted. Other tools used to identify demodex mites are dermatoscopy, trichograms, tape strips and microscopic examination of exudate. In rare cases, skin biopsies yield the diagnosis.

**Bacterial / mycobacterial infections.** Cytology should be applied in all cases of inflammatory alopecia and can reveal a definite diagnosis of pyoderma. Impression smears are performed by gently pressing a glass slide onto the affected skin. Bacteria and inflammatory cells on the skin surface, within a pustule or underneath a crust can be assessed by this method. Fine needle aspirations are used for nodular lesions caused by folliculitis or furunculosis.

If the clinical findings suggest the presence of deep inflammation sterile biopsies for microbial tissue culture should be obtained. To avoid the need to rebiopsy, biopsy specimens for histological examination should be taken simultaneously. For microbial culture, surgical preparation of the biopsy site and the sterile collection of the biopsy is important to avoid contamination with surface bacteria of the sample. It is one option to cut 8mm punch biopsies in half. Care has to be taken that the biopsies are cut in the direction of hair growth so that longitudinal sections of hair follicles can be examined histologically. One-half is fixed in formalin for histopathology; the other half is sent to a laboratory for microbiological culture. For the latter purpose, the epidermis is best cut off to avoid further contamination from superficial bacteria. For shipping, the sample for microbiological culture is placed in a small sterile bottle with either culture medium or saline. The samples should be examined for aerobic and anaerobic bacteria. Further special cultures have to be discussed with the laboratory depending on the suggested type of infection.

**Malassezia infections.** Cytology of direct impressions with a clear sticky tape or glass slide are useful for the detection of *Malassezia* spp.

**Surface mites.** Surface mites such as *Sarcoptes* and *Cheyletiella* can cause extensive pruritus and secondary alopecia due to skin inflammation and excessive hair breaking through scratching. Superficial skin scrapings are helpful to diagnose surface mites such and should include a large area of skin. *Cheyletiella* can also be found by collecting hairs and scales with a flea comb or tape strip.

**Leishmania infection.** In Leishmania infections, the inflammatory infiltrate often effaces the hair follicle and vasculitis is seen frequently. Both may result in alopecia. Diagnosis is made with impression smears of crusty lesions, serology, fine needle aspirates of skin, lymph nodes or bone marrow, biopsies or PCR.
Further assessment of inflammatory alopecia without infectious cause
There are several immune-mediated diseases causing inflammatory alopecia. These include alopecia areata, pemphigus foliaceus, drug reactions, idiopathic mural folliculitis and/or perifolliculitis, sebaceous adenitis and eosinophilic folliculitis. Cytology of pustules or crusts of pemphigus foliaceus can show acantholytic cells or in the case of eosinophilic folliculitis large numbers of eosinophils. Large follicular casts, keratin cuffs surrounding several normal hair shafts extruding from one single orifice, may be helpful to diagnose sebaceous adenitis. However final diagnosis of sebaceous adenitis and the other diseases requires biopsies. It has to be noted that follicular casts may also be present in demodicosis or endocrinopathies. Hypersensitivity reactions result in severe pruritus and secondary alopecia. Ischemia is another cause for alopecia. It may be caused by inflammation of vessel walls or by vasculopathy. In both cases skin biopsies are the adequate diagnostic tool.

Further assessment of non inflammatory alopecia
As outlined before, non inflammatory alopecia may have a hereditary cause or be acquired. The information received from the signalment and the history are completed by several laboratory examinations.

Trichography. Trichography is a valuable diagnostic tool to examine hair shaft changes in cases of follicular or hair shaft dysplasia. Small fragile hair shafts with an irregular outer contour indicate an impaired hair shaft formation which may result in hair that breaks easily once outside the follicle (but can occur also in inflammatory alopecia). Pili torti, trichoptilosis, trichorhexis and other trichomalacias have been diagnosed in trichograms. Hair shafts with huge melanin aggregates disrupting the hair shaft cuticle and misshaped hair bulbs are seen in colour dilution alopecia or black hair shaft alopecia.

Trichograms are only partially helpful to evaluate hair cycle disorders. Hair cycle disorders are histologically characterized by an increased number of hairless (kenogen) follicles and a reduced number of anagen follicles. Since kenogen can not be assessed by trichogram and the percentage of anagen and telogen follicles may vary with dog breeds and season a reduced number of anagen follicles in the trichogram may only allow the suspicion of a hair cycle disorders.

Blood tests and urinalysis. These tests are applied if internal or endocrine disease are suspected. Specific dynamic tests of endocrine function aid to diagnose hair cycle disorders of endocrine origin.

Selected References
DEMODICOSIS – IS IT STILL A PROBLEM?

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Introduction / Pathogenesis

*Demodex canis* is an obligate parasite of the dog and low numbers of mites are part of the normal cutaneous fauna. Within the first days after birth, the mites are transmitted from the bitch to the nursing puppies. Transmission of clinical disease from dogs with generalized demodicosis to normal dogs is typically not seen and the disease is not considered contagious. Fusiform eggs of *Demodex* mites hatch into six-legged larvae, molting into eight-legged nymphs and finally maturing into adults. In the dog, *Demodex canis* is the most commonly recognized mite. However, a short-bodied mite seems to inhabit the stratum corneum; this mite is supposedly of the same species as *D. canis*. A long-bodied mite was first reported to cause oily skin and subsequently characterized and named *D.injai*. It resides in the pilosebaceous unit.

When considering the pathogenesis of demodicosis in dogs, it is important to distinguish between juvenile onset and adult onset disease as well as between localized and generalized forms. The differentiation between localized and generalized disease is sometimes difficult. Localized demodicosis usually heals spontaneously. Generalised disease may also resolve spontaneously, but studies to evaluate the approximate rate of self-cure are lacking. With juvenile onset, certain breeds are at risk and cessation of breeding with affected animals reduces if not eliminates juvenile generalized demodicosis from breeding kennels. Other predisposing factors mentioned in the literature include short hair, poor nutrition, stress, oestrus, endoparasites and debilitating disease. Initially, an immunodeficiency was postulated as a reason for the development of demodicosis. Supporting this pathogenesis, more recent studies show a decreased CD4+ T-lymphocyte count, a lower CD4/CD8 ratio, a significantly decreased IL-10 concentration in dogs with recurrent demodicosis and an increased apoptosis of peripheral blood mononuclear cells. If these changes are a consequence of the proliferation of the *Demodex* mites or a previous change predisposing to this proliferation is not known at this point. However, a high number of dogs with juvenile demodicosis will be cured and do not develop the disease again.

Drugs or diseases altering the immune response may be the triggers for adult-onset demodicosis. Hypothyroidism, hyperadrenocorticism and leishmaniasis have all been reported as predisposing factors to adult-onset demodicosis. Glucocorticoid therapy, neoplasias or chemotherapy have all been reported as well, but only based on anecdotal evidence. Idiopathic adult-onset demodicosis also exists. In cats, demodicosis is typically due to an underlying systemic disease!

Clinical signs

Clinically, canine demodicosis is characterized initially by erythema, papules and comedones, as mites accumulate in the hair follicles. Alopecia and scaling may also be seen. Later on, influx of inflammatory cells may lead to pustule formation. With severe disease, follicles rupture and furunculosis with deep lesions and crusting develops. Lesions can occur anywhere on the body, although the face and feet are most commonly affected. Certain breeds are predisposed for
demodicosis, typically short coated breeds such as Chinese Shar Pei, Staffordshire terrier or bullterrier.

**Diagnosis**
Diagnosis is made by deep skin scrapings, trichogram or tape squeezes. A deep skin scraping is one of the most common diagnostic procedures performed in veterinary dermatology. A small area of affected skin (1-2 cm²) is scraped in the direction of hair growth until capillary bleeding is observed. A blade covered with mineral oil should be used. Lesions such as follicular papules or pustules are good sites for scraping. Because *Demodex* mites live in the hair follicles, it is useful to squeeze the skin prior to and during the scraping in an attempt to push the mites out from the depths of the follicles. Paws and faces are difficult to scrape and skin biopsies may be needed in some of these patients to confirm the diagnosis. Some breeds such as Shar Peis with demodicosis are anecdotally reported to be negative on scrapings and may have to be biopsied for diagnosis. Although *Demodex canis* is a normal part of cutaneous fauna and thus an occasional mite can be found on skin scrapings of normal dogs, it is extremely rare to see more than one mite on a dog not affected by demodicosis. If only one mite is found, further scrapings or a biopsy are recommended. When evaluating deep skin scrapings, it is important to assess and to note in the record the site of scraping and the relative numbers of adults, larvae, nymphs and eggs per microscopic field. In subsequent visits, assessment of response to therapy relies on the comparison of such numbers. Scrapings should be repeated at the same sites monthly when monitoring patients with demodicosis. More recently, an acetate tape impression with skin squeezing was found to be more sensitive than deep skin scrapings in some studies, but not in others.

**Treatment**
Treatment for generalized demodicosis may include a number of medications, many of which are not registered for that indication anywhere in the world.

*Amitraz.* Amitraz is a miticidal rinse. The adverse reactions associated with amitraz administration or application resemble those induced by alpha 2-adrenergic agonists such as xylazine. These are sedation, bradycardia, hypothermia, hypotension, bloat, polyuria, vomiting and hyperglycemia. Yohimbine at 0.1mg/kg IV antagonizes the CNS-depressant and bradycardic effects of amitraz. Amitraz is registered for the treatment of canine demodicosis in many countries. Clipping the entire dog is essential to allow better contact of amitraz with the skin. All crusts should be removed (preferably by shampooing with an antibacterial follicular flushing agent such as benzoyl peroxide). The dog has to be dry completely, before being sponged down with amitraz. The treating person should wear protective gloves and work in a well-ventilated area. Owners with asthma are advised to find somebody else for the rinses. The dog should stand in a tub with its feet in the amitraz solution to allow soaking of the often extensively affected feet. Amitraz causes a transitory sedative effect for 12 to 24 hours. Concentration of the drug and frequency of application influences the response rate. The author uses a concentration of 600 ppm once to twice weekly. In patients with demodicosis, the procedure should be repeated until 4 weeks after two successive skin scrapings (2-4 weeks apart) fail to reveal live mites. Treated dogs should not get wet or be washed. A recent study showed a quicker response, when an inactivated parapoxvirus ovis preparation was injected initially concurrently to
treatment with amitraz. However, the study included only a small number of dogs and further studies confirming those findings are needed.

**Ivermectin.** Ivermectin orally at 300 - 500 mcg/kg daily is used in the treatment of demodicosis with good success. It must not be used in collies and Old English sheep dogs, as it commonly causes adverse reactions in these breeds. Ivermectin paralyzes nematodes and arthropods by potentiating gamma - aminobutyric acid (GABA), binding to its receptor and stimulating GABA release. In mammals, GABA is only found in the CNS and ivermectin does not readily cross the blood brain barrier. However, in some breeds adverse reactions are seen commonly and include ataxia, mydriasis, tremors, stupor, salivation, bradycardia and respiratory arrest. These side effects are seen in Collies at a dose between 100 mcg/kg and 200 mcg/kg due to a genetic defect in the MDR-1 gene. Other breeds may be affected as well showing ataxia and tremors at lower doses. However, there are a number of dogs with adverse effects to ivermectin and an intact MDR-1 gene, alternative mechanisms thus are possible. Thus, the routine protocol for a dog that did not receive ivermectin before, is a slow increase from 50 mcg/kg to 100 mcg/kg to 150 mcg/kg to 300 mcg/kg on subsequent doses every day. The owners should monitor the animal carefully for the above mentioned side effects. If any signs of ataxia or tremors occur, administration of the drug must be discontinued immediately. Once the maintenance dose is reached, demodectic patients receive that dose once daily until 4 weeks after the second consecutive negative monthly skin scraping. With daily dosing, the serum level increases for weeks due to the long half-life of ivermectin. Thus, patients need to be monitored for side effects for the first 8 weeks.

**Moxidectin.** Moxidectin is another milbemycin that was evaluated for the therapy of canine generalized demodicosis. Studies have evaluated moxidectin at 200-400 mcg/kg/day orally, two of which employed the initial gradual dose increase advocated for ivermectin. Reported side effects were ataxia, lethargy, inappetence and vomiting. As moxidectin is a macrocyclic lactone and has a similar mode of action to the other drugs in this group, the success rate and rare adverse effects are not surprising. However, more studies with longer follow-up period periods are needed to identify potential benefits and disadvantages of this drug. Moxidectin became recently available as a spot-on formulation approved for the treatment of canine demodicosis. Studies have shown increasing efficacy with more frequent application of the spot-on, but the success rate in dogs seems to be lower than that seen with oral macrocyclic lactones.

**Doramectin.** Doramectin is the final macrocyclic lactone successfully used for therapy of generalized demodicosis. In one study, 23 dogs were injected weekly with 600 mcg/kg subcutaneously. Ten of the dogs were cured, 7 relapsed after 1-24 months (2 of which responded to repeat doramectin treatment) and 6 were lost to follow-up. None of the animals in this study showed any adverse effects with therapy. A more recent large study in general practice revealed a very high success rate with a good safety profile in first opinion cases. A further study comparing twice weekly and once weekly application did not find a difference between the two protocols.

**Isoxazolines.** Currently, the most exciting drugs for the treatment of demodicosis are the isoxazolines. Studies have been presented and published showing effects of fluralaner, afoxolaner, lotilaner and sarolaner against generalized demodicosis. All of those drugs seem to
work well for canine demodicosis, with adverse effects being very rare, resolution of clinical signs achieved fairly quickly and remission rates high. Rarely, some dogs do not respond to these types of drugs. It is unknown if that is due to resistance of the mite or malabsorption of the drug.

Treatment of an underlying primary disease may result in resolution of the demodicosis. This emphasizes the need for pursuing and treating concurrent diseases in patients with adult-onset demodicosis. Although secondary bacterial infections were treated with antibiotics in the past, more recent evidence questions the need for oral antibiotics in dogs with demodicosis. In one study, dogs with generalized demodicosis treated with oral macrocyclic lactones and antibacterial shampoos responded as quickly and as completely as dogs treated with additional oral antibiotics. This indicates that most dogs do not need oral antibiotics if the demodicosis is treated with appropriate miticidal agents.

When dogs do not respond to a given treatment, changing the therapeutic agent leads to remission in two-thirds of dogs. Similarly, if a relapse occurs after initial remission, a second treatment attempt (either with the same or a different drug) will lead to resolution in approximately two-thirds of dogs.

**Selected References**
Clinical Consensus Guidelines of the World Association for Veterinary Dermatology “Diagnosis and Treatment of dermatophytosis in dogs and cats” by Dr Karen Moriello et al were published in the 2017 Veterinary Dermatology journal and also published with free access online on the World Association for Veterinary Dermatology website https://wavd.org/continuing-education/consensus-guidelines. Readers are directed to make use of this excellent resource.

**Diagnosis**

In the Clinical Consensus Guidelines (CCGWAVD) no one diagnostic test was identified as the gold standard. Diagnosis accuracy is dependent on the stage of infection, presence or absence of treatment, sampling technique, site selection, clinician training, quality of the tool and ability to examine the animal. The most important question to answer is which tests confirm the presence of an active infection so an informed decision can be made regarding treatment.

Woods lamp and direct examinations were reported to have good positive and negative predictability. Dermatophytosis is diagnosed by using a number of complementary diagnostic tests, including Woods lamp and direct examination to document active hair infection, dermatophyte culture by toothbrush technique to diagnose fungal species involved and monitor response to therapy, and biopsy with special fungal stains for nodular or atypical infections.

**History and signalment**

A common clue as to the possible presence of dermatophytosis due to *Microsporum canis* is a history of a new kitten in the household, or in the case of *Trichophyton* spp., a knowledge of rodent chasing behavior in a terrier type dog. Yorkshire terrier dogs may be predisposed to superficial dermatophytosis and subcutaneous dermatophytic infections, most commonly due to *M. canis*. Working and hunting dogs may be at increased risk of exposure to geophilic dermatophytes. Immunosuppression with certain drugs such as ciclosporin may raise the index of suspicion for infectious disease including dermatophytosis in cases with a consistent clinical picture.

Review of prevalence studies in the CCGWAVD identified that dermatophytosis is an uncommon skin disease and that the most at-risk populations are young animals (puppies), free roaming animals and those living in warm locations. The incubation period between inoculation and development of clinical lesions is usually 7 to 21 days.

**Clinical signs**

Dermatophytes invade keratinized structures and infection with dermatophytes is most commonly follicular in dogs. The most consistent clinical sign is single or multifocal circular patches of peripherally expanding alopecia with variable scaling. Some patients may have the classic ring-shaped lesion with central healing, fine follicular papules and peripheral crusts.
Variations in clinical presentation reflect the variation in the host-fungus interaction and the degree of inflammation. Pruritus is variable but usually minimal or absent.

Typically lesions are asymmetrical but sometimes symmetric nasal or facial folliculitis and furunculosis may be mistaken for auto-immune skin diseases such as pemphigus foliaceus. Pustular dermatophytosis has been described in dogs and may also mimic pemphigus foliaceus clinically. *Trichophyton* infections may affect the non-follicular skin including the nasal planum, footpads and or nails.

Dogs can develop nodular dermatophyte infections (kerions, pseudomycetomas, mycetomas). A dermatophyte kerion is a single or multiple erythematous alopecic dome shaped, exudative nodular type of furunculosis that may develop multiple draining tracts and most commonly appears on the face or distal limbs.

**Woods Lamp**

The Woods lamp is an ultraviolet lamp. The characteristic green yellow fluorescence observed in hair shafts in 50% or more of *M. canis* infections is due to a water-soluble chemical metabolite (pteridine) located within the cortex or medulla of the hair. Fluorescing hairs are most likely to be found in untreated infections. False positive and false negative results are due to inadequate equipment, lack of magnification, patient compliance, poor technique or lack of training. Be sure to observe the fluorescence in the hair shaft itself; false positives are common when topical medications or skin scale rather than the actual hair itself seemingly fluoresce. These false positives lack the apple/emerald green true fluorescence of *M. canis*.

**Dermoscopy**

Dermoscopy uses illuminated magnification to examine skin and hair and is commonly used in human medicine. In one study in cats, dermoscopy showed comma hairs and hairs with a corkscrew or coiled appearance typical of fungal invasion in people; similar findings are likely in dogs.

**Microscopic examination of plucked hair, scraped scales, tape impressions of skin**

Microscopic examination may reveal hyphae and arthrospores in 40 to 70% of cases and provide rapid and definitive evidence of dermatophytosis. This test is easy to perform and it is recommend that all clinicians should become familiar with it.

**Fungal culture**

Fungal culture of affected hair and scales is the most reliable diagnostic test and is the only way to identify the specific dermatophyte. However, false positives may be obtained if the animal is a carrier or has recently been exposed to a contaminated environment. A false negative result is also possible (e.g., when the microscopic examination of hairs is positive but the culture is negative).

Three sampling techniques have been described in the literature: hair coat brushings with a sterile toothbrush or square of carpet, hair plucking from the margins of lesions and sticky tape sampling. There is no standard technique and 20 brush strokes, 2 to 3 minutes of brushing or brushing until the bristles are full of hair are all sampling end points.
The dermatophyte test medium (DTM) is a nutrient growth medium containing cycloheximide, gentamicin and chloramphenicol to suppress bacterial and contaminant fungal overgrowth, and phenol red, a color indicator to aid in the early recognition of possible dermatophyte species. The color change in the medium from yellow to red is the result of a pH change triggered by fungal growth. It does not matter if the DTM is incubated in dark or light. When a colony grows and a color change is observed, the fungal colonies should be examined under a microscope. The DTM should be monitored daily and growth recorded once a week. Although dermatophyte test medium is widely used and recommended for culturing dermatophytes, some mycologists believe it to be inferior to Sabouraud dextrose agar.

*Microsporum canis* growth is rapid, woolly or cottony; the colony is white to yellowish and flat with radiating edges. There are abundant large macroconidia, which are thick walled, spindle shaped with 6 or more compartments with a shiny surface and a terminal knob. The microconidia are slender.

*Trichophyton mentagrophytes* has a flat colony with a cream yellow powdery to granular surface. Small spherical microconidia are produced in grape like clusters. Macroconidia may be present in variable numbers and are cigar shaped, smooth and thin walled. There is an abundance of coiled spirals in many strains.

*Microsporum gypseum* has sparse arthroconidia in chains. There is a rapid spreading flat thallus with the texture of chamois and a powdery surface. Macroconidia are present in enormous numbers and are symmetrical, ellipsoid, echinulate and thin walled, with up to 6 compartments.

Toothbrush cultures commonly remain positive until 1 to 2 weeks after clinical resolution.

**Histopathology**
Biopsy findings are as variable as clinical lesions and this test is not as sensitive as culture. However, it can provide a faster answer. When the true significance of a culture is questioned, the demonstration of fungal organisms in biopsy specimens is definitive proof of true infection. Histopathology is most useful in nodular forms of dermatophytosis, ie the kerion and pseudomycetoma. It may be impossible to culture the organism in such cases by collecting hair and scales. Routine H&E staining may not identify dermatophytes and special stains such as periodic acid Schiff (PAS) and Grocott methenamine silver (GMS) are needed.

**Immunohistochemistry, PCR, DNA fingerprinting**
PCR detection of dermatophyte DNA can be helpful. However, a positive PCR does not necessarily indicate active infection, as dead fungal organisms from a successfully treated infection will still be detected on PCR, as will non infected fomite carriers.

Immunohistochemical methods are being explored for usefulness in confirming diagnosis of dermatophytosis. Detection of chitin synthase 1 gene appears to be rapid and specific for dermatophytes.
Molecular typing may be useful not only for diagnosis but also for tracing the epidemiological spread of various strains of dermatophytes. One study using a random amplified microsatellite inter single sequence repeat PCR method found 21 genotypes among 24 isolates of *M. canis*. This demonstrates a high degree of polymorphism among different strains of *M. canis*.

**Treatment**

The Clinical Consensus Guidelines (CCGWAVD) state that successful treatment of dermatophytosis requires concurrent use of systemic oral anti-fungals and topical disinfection of the hair coat. Systemic anti-fungal drugs were reported to have a wide margin of safety and physical cleaning was noted to be most important for decontamination of exposed environments.

*Microsporum* infections tend to spontaneously resolve in healthy dogs by around 3 months after inoculation. Animals with generalized dermatophytosis usually require therapy. In general, *Trichophyton* infections do not spontaneously resolve and require therapy.

The goals of therapy are to treat the patient as well as in-contact animals and also to reduce contagion to the environment. Shortening the course of the disease reduces spread to other animals and people. The Clinical Consensus Guidelines (CCGWAVD) state that negative PCR in a treated animal, and negative fungal culture from an animal with no lesions and a negative Woods lamp is compatible with cure. The Guidelines also make the point that confinement of animals affected with dermatophytosis needs to be used with care and for the shortest time possible. Dermatophytosis is a curable disease, but behavior problems and socialization problems can be lifelong if young or newly adopted animals are not socialized properly.

*Treatment of the patient*

Clipping the coat, particularly of long haired dogs, helps to rid the animal and the environment of infected hairs. Clipping of the hair coat sometimes leads to worsening of clinical signs within 7 to 10 days after clipping. This illustrates the importance of skin trauma in the spread of infection. Clipping should be done gently, using scissors or a clipper blade that does not clip too short, to avoid traumatizing the skin. Clipped hair should be collected and disposed.

Topical therapy using a miconazole/chlorhexidine shampoo twice weekly and cream or lotions containing clotrimazole, miconazole and enilconazole twice daily can be helpful as concurrent treatments but not as sole therapy. Lime sulfur and also enilconazole whole body treatments (in countries where this is available) twice weekly are currently recommended effective topical therapies. Most animals will benefit from systemic therapy.

Itraconazole, a first generation triazole, is the systemic drug of choice due to the high efficacy, ease of administration and low incidence of toxicity. It works by inhibiting fungal cytochrome P450 enzyme 14-alpha demethylase to prevent the conversion of lanosterol to ergosterol. Capsules are recommended to be administered with food to decrease gastrointestinal adverse effects and to decrease gastric pH and enhance absorption. It is not recommended for use in pregnant or nursing dogs. Fluconazole and ketoconazole are less expensive than itraconazole but are less effective and ketoconazole is not available in many countries.
Terbinafine is an allylamine antifungal that inhibits the enzyme squalene epoxidase and is fungicidal against dermatophytes. This is reported to be an effective and safe treatment for dermatophytosis although it is not licensed for use in small animals. Its mode of action does not affect mammalian cytochrome P450.

Griseofulvin is effective but may be expensive and has the potential for side effects including gastrointestinal upset and myelosuppression. Griseofulvin is a teratogen and should not be used in pregnant animals. Anti-fungal vaccines do not protect against challenge exposure but may be a useful adjunct therapy. Lufenuron is not effective against dermatophytes and has no place in the treatment of dermatophytosis.

Prognosis is good for dogs with kerion reactions. In dogs with pseudomycetoma or mycetoma, the treatment of choice is surgical excision and concurrent systemic antifungal treatment; prognosis is guarded.

Treatment of the environment
Clients should be advised to treat the environment as well as the dog. This minimizes the risk of disease transmission to people and other animals and minimizes fomite carriage on the hair coat of animals that can complicate monitoring of disease.

Arthrospores in hair shafts can contaminate the environment for up to 24 months. The highest environmental contamination has been found in households with infected kittens. Spores are easily spread by air currents, contaminated dust, cleaning utensils and movement between rooms.

Care must be taken in households with humans at higher risk of infection, including children, the elderly and immunocompromised people such as those with immune mediated diseases or undergoing chemotherapy for example.

It may be helpful to confine culture-positive animals to a room or area in the house which is easily cleaned, and people entering and leaving the room can then wear protective clothing and change clothing when entering or leaving the room. Disposable covers may be used on shoes, and hands may be washed and sanitized when moving between rooms.

All non-porous surfaces should have debris removed by thoroughly vacuuming and sweeping, washed with a detergent until visibly clean, rinsed and then disinfected. Rugs and fabrics that cannot be destroyed or removed should be vacuumed and if possible washed with an anti-fungal disinfectant. Steam cleaning of carpets has been recommended as a method of decontamination. To kill fungal spores the temperature of the water being forced into the carpets must be at least 43.3C. This is not usually achievable with home systems but steam cleaning and shampooing can be helpful.

Ideally anti-fungal disinfectants should have good anti-fungal efficacy, be non-toxic and have low irritancy to animals and users. Sodium hypochlorite (household bleach) has been consistently shown to be an effective disinfectant when used at concentrations ranging from 1:10 to 1:100 even with short contact times. It can fail if it is out of date. If household bleach is used it should be prepared at least once weekly and stored in a dark opaque container. Clients should
be warned that it can be irritant and can discolor and damage surfaces. Other options include enilconazole, accelerated hydrogen peroxide, potassium peroxymonosulfate, over the counter bathroom or general cleaners with label claims for fungicidal action and essential oils.
HYPOTHYROIDISM – WHAT SHOULD WE BE DOING?

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The true prevalence of canine hypothyroidism is not known, yet it is often described as the most commonly diagnosed endocrine disorder in the dog. It is a rare disease in the cat. Many dogs that are placed on thyroid replacement therapy with the diagnosis of hypothyroidism are in all likelihood not truly hypothyroid. This in part results from the fact that no one test is optimal, that drugs and concurrent illness can alter test results, and that the clinical signs of hypothyroidism can be vague and involve almost any organ system. So attaining the correct diagnosis can be challenging and hypothyroidism is deemed the cause for many presenting complaints. Making the diagnosis should be based on the presence of supportive clinical signs in the patient whose routine lab work does not reveal any other significant disease and evaluation of the thyroid gland function and documenting it as hypo-functioning.

Pathogenesis
Thyroid hormone deficiency can occur if there is dysfunction at any point in the hypothalamic-pituitary-thyroid gland axis. Over 95% of cases of canine hypothyroidism are the result of dysfunction of the thyroid gland itself. The causes of primary acquired hypothyroidism in the adult dog, based on histologic evaluation of thyroid gland biopsies, are equally divided between lymphocytic thyroiditis and idiopathic atrophy of the thyroid gland. Idiopathic atrophy of the thyroid gland may represent the end stage of lymphocytic thyroiditis. Lymphocytic thyroiditis is a slowly progressive disease and development of obvious signs of hypothyroidism will not occur until at least 75% of the function of the thyroid gland is lost. Certain breeds and dog leukocyte antigens (DLA) haplotypes are associated with increased risk for development of hypothyroidism. These breeds include the Doberman pinscher, giant schnauzer, Rhodesian ridgeback and the English setter.

Neoplastic destruction of thyroid tissue can be a rare cause of primary hypothyroidism, as can iodine deficiency, congenital hypothyroidism, treatment with radioactive iodine and certain drug therapies (potentiated sulfonamides).

Less than 5% of dogs with hypothyroidism have problems with the function of the pituitary gland to secrete thyrotropin (TSH) with resultant secondary follicular thyroid atrophy (secondary hypothyroidism) or problems with the hypothalamus to secrete TRH (tertiary hypothyroidism). Reported causes of secondary hypothyroidism include pituitary tumors, congenital pituitary malformations (cystic Rathke’s pouch), pituitary trauma or surgery.

Thyroid Hormone Physiology
Thyroid hormone regulation is a complex process that involves the hypothalamus, pituitary gland, thyroid gland, plasma transport proteins and the cellular uptake and metabolism of the thyroid hormones. Understanding physiology and regulation can help clinicians better interpret test results. Thyrotropin-releasing hormone (TRH) is produced by the hypothalamus and stimulates the secretion of TSH by the pituitary and also increases the bioactivity of TSH. TSH stimulates the synthesis and release of thyroxine (T4) and 3,5,3’-triiodothyronine (T3).
thyroid hormones once released have a negative feedback on the pituitary and hypothalamus to decrease both TSH and TRH secretion. TSH secretion also decreases because of somatostatin, dopamine, catecholamine, TNFα and some interleukins.

Once in the circulation, thyroid hormones are tightly bound to plasma transport proteins. In dogs, protein binding of T4 is weaker than in human beings and consequently dogs have lower serum total T4 levels and higher free T4 levels than are documented in people. Only the unbound hormone is “free” or available to enter cells via either passive diffusion or receptor mediated active transport. Once within the cell, T3 binds to nuclear receptors, initiating the actions of thyroid hormone on cellular protein synthesis. Thyroxine (T4) must undergo deiodination to T3 to be able to bind to the cell’s nuclear thyroid receptor. About 40% to 60% of T3 in the dog is derived from extra-thyroidal deiodination of T4 in peripheral tissues. Therefore T4, although it has some intrinsic activity, is often considered a prohormone requiring deiodination to T3 to exert the metabolic effects of thyroid hormone. Thyroxine can also undergo deiodination to form the metabolically inactive reverse T3 (3, 5’, 3’-triiodothyronine). This deiodination pathway increases during periods of nonthyroidal illness (NTI).

Clinical Signs
The clinical signs of hypothyroidism are varied and often vague and insidious in their onset. Thyroid hormones influence protein synthesis in most tissues and consequently the function of many organ systems can be altered in hypothyroidism. The most common clinical signs are referable to changes in overall metabolic rate and the appearance of the skin. These signs include lethargy, weight gain, exercise intolerance. alopecia, changes in coat quality and disturbances in cornification. Some clinical signs reportedly associated with hypothyroidism may result from breed predispositions to both hypothyroidism and other disorders rather than a cause and effect due to hypothyroidism. Less common clinical signs reportedly associated with hypothyroidism include muscle weakness from myopathy or peripheral neuropathies, CNS signs associated with myxedema, prolonged anestrus, prolonged parturition and increased periparturient mortality of pups, and corneal lipid accumulation.

Dermatologic Signs: 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 glycosaminoglycan in the dermis. Clinically, this results in alopecia (often in areas of wear: collar region, tail), a dull, dry hair coat, variable hyper pigmentation, scaling and myxedematous changes that give the “tragic look of hypothyroidism. The normal barrier function of the epidermis is likely impaired in hypothyroid animals and in animal models, impaired neutrophil and lymphocyte function has been reported. Consequently, recurrent pyoderma and otitis externa can occur in hypothyroid animals.

Spontaneous hypothyroidism in cats is rare. One reported case had similar clinical signs to dogs with a dull dry, hair coat 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. A recent study identified seven cats with spontaneous hypothyroidism with six having bilateral goiter and four had hair coat changes.
Diagnosis of Canine Hypothyroidism

Clinicopathologic Findings: If there is clinical evidence to make the clinician suspicious of the diagnosis of hypothyroidism then a complete blood cell count (CBC), serum biochemistry panel and serum T4 level should be performed. The documentation of a mild, normocytic, normochromic anemia and fasting hypercholesterolemia will add evidence for the suspicion of hypothyroidism. These tests are equally important to evaluate for any evidence for non-thyroidal illness (NTI). NTI and certain medications (corticosteroids, phenobarbital, sulfa antibiotics, some NSAIDs) can influence thyroid levels and complicate the interpretation of thyroid tests and the correct diagnosis of hypothyroidism. Dogs with moderate or severe NTI, including dogs with hyperadrenocorticism, will have total T4 is decreased below normal range. Thyroid testing of sick dogs, or dogs receiving medications known to alter thyroid hormone test values should be delayed until the non-thyroidal illness can be resolved. Drugs known to alter thyroid values should be discontinued for several weeks prior to obtaining tests to evaluate thyroid function.

Thyroid Function Tests: There are a number of different tests that can be considered when evaluating thyroid function but the most commonly used are baseline total thyroxine, (T4), endogenous TSH, free thyroxine (fT4) and thyroglobulin autoantibodies. A challenge in interpretations is that there a number of factors that can influence measurable concentrations of thyroid hormones. These include age, breed, sex hormone levels, athletic training of the dog, NTI and a variety of drugs (see factors affecting thyroid testing below).

Serum Total Thyroxine (T4) Concentrations: Total thyroxine (T4) levels will be low in dogs with hypothyroidism. A single determination of serum total T4 concentration can be used as a screening test but is most useful if the value is greater than 2 mg/dl as this will eliminate or “rule-out” the diagnosis of hypothyroidism. Dogs with low or low normal T4 levels with supportive clinical signs of hypothyroidism disease (with no evidence for NTI and no drug history to influence T4 values) need to undergo further testing to confirm the diagnosis. A thyroid panel, which includes TSH and fT4, is the most common next diagnostic evaluation to pursue. It is critical to remember that depending on the severity of concurrent NTI euthyroid dogs may have extremely low serum levels of T4. Older dogs and sight hounds (any age) have lower T4 concentrations. Numerous drugs can change T4 values.

Serum Total T3 Concentrations: Although T3 is the most important biologically active thyroid hormone at a cellular level, a large portion of it is produced by deiodination in peripheral tissues. It is therefore not the predominant circulating hormone produced by the thyroid gland and T3 measurements are unreliable when used to evaluate thyroid gland function. It is not considered a sensitive or specific test for diagnosing canine hypothyroidism.

Free Thyroxine (fT4): Free T4 (fT4) is the biologically active form of thyroxine. It regulates pituitary production of TSH. The fT4 fraction of thyroid hormone is less influenced by NTI and drug therapy than total T4 concentrations. However, it can be decreased by glucocorticoids, long-term anticonvulsants and trimethoprim-potentiated sulphonamides. Commercial assays that measure fT4 by radioimmunoassay are less accurate in dogs. Using an equilibrium dialysis assay, fT4 concentrations measurements are more accurate and this is the preferred method to
measure fT4 in the dog. The sensitivity of measuring fT4 to diagnose hypothyroidism has been reported to be 80 to 98% and the specificity has been reported to be between 92 to 98%.

**Endogenous Canine TSH:** In people, hypothyroidism and hyperthyroidism are accurately diagnosed using sensitive assays for endogenous TSH. TSH is a species specific hormone and until 1997 a commercial assay for measuring endogenous canine TSH (cTSH) had not been validated. TSH should be elevated in hypothyroid animals but it is not consistently increased in canine hypothyroidism; up to 38% of hypothyroid dogs may have a cTSH concentration within the reference range. TSH can also be elevated in some dogs with NTI and inappropriate elevations have been documented in 18% of euthyroid dogs. The use of this assay as a screening test is questionable since the sensitivity is low; it does however have good specificity. It should not be used alone but it does increase the specificity of fT4 measurements. Measuring the cTSH response to TRH administration did not improve the accuracy of diagnosing hypothyroidism.

**Free Thyroxine (fT4) and Endogenous TSH Serum Concentrations:** The test of choice to confirm the diagnosis of hypothyroidism is to concurrently measure the serum concentration of endogenous canine TSH and free T4 concentrations (measured by equilibrium dialysis). These two tests used together have a reported accuracy of 86%, a sensitivity of 74 to 80% and a specificity of 98%. An elevated cTSH serum level and a decreased fT4 concentration are consistent with hypothyroidism.

**TSH Stimulation Testing:** Previously, this test was the gold standard for the diagnosis of hypothyroidism. It evaluates thyroid gland reserve and is used in dogs with low T4 to differentiate hypothyroidism from NTI. Human recombinant TSH can be used in dogs to perform a TSH response test to evaluate for hypothyroidism. However, the human recombinant TSH, Thyrogen (Genzyme Corporation, Cambridge, MA, USA) is very expensive. If doses are kept in the freezer after reconstitution, it will provide TSH for 7 to 15 tests depending on the dose used. Serum T4 levels are measured before and 6 hours after intravenous administration of 75 to 150 micrograms of human recombinant TSH. Hypothyroidism is confirmed if the baseline and post stimulation T4 samples are below reference range.

**Measurements of Anti-Thyroglobulin, Anti-T4 and Anti-T3 Antibodies:** Antibodies to thyroglobulin, T4 and T3 can develop in lymphocytic thyroiditis. Anti-thyroglobulin antibodies were detected in 59% of hypothyroid dogs in one study. Dogs with positive anti-thyroglobulin autoantibodies but normal thyroid function do not require therapy but should be monitored for future development of hypothyroidism. Antibodies to T3 occur more commonly than anti-T4 antibodies and can interfere with some assays used to measure T3 or T4, resulting in an erroneously high value. When serum T3 or T4 values are elevated and do not correlate with the clinical presentation, evaluating for the presence of antibodies against T3 or T4 can help determine if there is interference with the assay.

**Factors Affecting Thyroid Function Testing**

**Nonthyroidal Illness:** Nonthyroidal illness can be caused by systemic illness (neoplasia, diabetic ketoacidosis, renal or hepatic disease, heart failure or severe inflammatory disease), surgery or trauma. NTI influence thyroid physiology and complicate the interpretation of thyroid tests and therefore the diagnosis of hypothyroidism. Studies have shown that 60% of euthyroid dogs with
severe illness will have low serum total T4 concentrations. Reductions in serum total T4 concentrations in severe illness correlate with a higher mortality rate. Reductions of thyroid hormones in NTI may serve a protective role against the catabolic effects of illness and it is not considered appropriate to supplement these patients with thyroid hormones. Possible mechanisms for the reduction in serum total T4 seen in dogs with NTI include impaired binding to, reduced concentrations of or reduced affinity for plasma carrier proteins. Decreases in TRH or TSH secretion resulting in decreased T4 production and possible direct effects on the thyroid gland suppressing T4 production are suspected to occur in NTI including hyperadrenocorticism. In dogs with severe NTI, serum total T4 is typically decreased, free T4 may be normal but can be decreased, and serum T3 concentrations are often low. TSH concentrations may occasionally be increased but are usually normal. Even the response to TSH stimulation can be blunted in NTI making the distinction between hypothyroidism and NTI difficult. The most accurate method to evaluate for hypothyroidism in a dog with concurrent severe NTI is to resolve the nonthyroidal illness and then assess thyroid function.

Effects of Drugs on Thyroid Tests: Glucocorticoids, phenobarbital and trimethoprim potentiated sulphonamide antibiotics are known to influence thyroid function tests. The effect of glucocorticoids on thyroid function testing is in part dosage dependent. Immuno-suppressive dosages of glucocorticoids produce greater suppression than anti-inflammatory dosages. Duration of therapy with glucocorticoids may also influence the degree of change to thyroid function. It is recommended that dogs not receive glucocorticoids for at least 4 weeks prior to evaluating thyroid function. Dogs receiving high doses for long periods of time may need longer withdrawal times. Dogs receiving phenobarbital have been documented to have decreased tT4 and decreased fT4 concentrations. Endogenous TSH was increased in one study compared to untreated epileptic dogs but was not significantly changed in another study evaluating the effects of anticonvulsants on thyroid function. Sulfur antibiotics can depress T4 secretion from the thyroid glands and cause functional hypothyroidism and consequently negative feedback to the pituitary gland is lost so endogenous TSH increases.

### Drugs

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Total T4</th>
<th>Free T4</th>
<th>Endogenous TSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucocorticoids</td>
<td>↓ or NC</td>
<td>↓ or NC</td>
<td>NC or ↓</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>↓ or NC</td>
<td>↓ or NC</td>
<td>NC or ↓</td>
</tr>
<tr>
<td>Sulfonamides</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td>Potassium Bromide</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Clomipramine</td>
<td>↓</td>
<td>↓</td>
<td>NC</td>
</tr>
<tr>
<td>Aspirin</td>
<td>↓</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>↓</td>
<td>NC</td>
<td>NC</td>
</tr>
<tr>
<td>Carprofen</td>
<td>↓ or NC</td>
<td>↓ or NC</td>
<td>↓ or NC</td>
</tr>
<tr>
<td>Nonthyroidal illness</td>
<td>↓</td>
<td>↓ or NC</td>
<td>NC or ↑(slight)</td>
</tr>
</tbody>
</table>

NC= no change

**Therapeutic Trials with Levothyroxine**
If the clinical suspicion of hypothyroidism is high and the diagnostic screening tests are equivocal it has been advocated to treat without a definitive diagnosis. This is in part because there is minimal risk to supplementing a normal dog with levothyroxine and the medication is
relatively inexpensive. Thyroid hormone therapy is a signal for hair follicles to initiate anagen so hair regrowth may occur even in a dog with NTI. To confirm that a dog’s clinical signs were caused by hypothyroidism rather than a thyroid hormone responsive change, thyroid supplementation should be stopped after there has been a positive response to therapy and clinical signs should return.

It is important to remember that once levothyroxine is administered to a normal dog the pituitary will stop secretion of TSH because of negative feedback from the exogenous source of thyroid hormone. This will result in atrophy of the thyroid glands. To be able to evaluate thyroid function once thyroid supplementation was initiated will require that the levothyroxine is discontinued for at least 6 to 8 weeks. An incorrect diagnosis of hypothyroidism can be costly as therapy is life long and it could also mean the delay in diagnosing another disease.

**Treatment and Monitoring**
Levothyroxine administration (0.01-0.02 mg/kg orally twice daily) is required lifelong therapy to manage canine hypothyroidism. Some dogs can be managed with once a day therapy (0.02 to 0.04 mg/kg), with a maximum initial dose of 0.8 mg. Ideally, dogs should receive the same brand of replacement thyroid hormone. There can be differences in the clinical response of dogs when receiving different manufactured sources of thyroid supplementation. Iatrogenic hyperthyroidism is uncommon but is more common to occur in large breed dogs. For this reason it is advocated by some to use a dosage protocol of 0.5 mg/m2 for large breed dogs.

Therapeutic success should be judged based on clinical response and evaluation of serum T4 levels after a steady state is attained. Typically, a steady state is reached 7 to 10 days after initiating therapy. It may take weeks to months to have resolution of clinical signs referable to the initial hypothyroid state. After 6 to 8 weeks, the clinician should critically evaluate the clinical response. A post-pill level 4 to 6 hours after oral administration of the levothyroxine provides information about the peak T4 level induced by the replacement therapy. A high normal to elevated T4 level is expected if the dog is receiving adequate supplementation. If TSH is concurrently measured, it should be normal and if previously elevated it should have decreased. A trough T4 level, just prior to the administration of the dose of levothyroxine is recommended in dogs receiving once daily administration of levothyroxine and serum T4 and fT4 by equilibration dialysis should both be in normal range if adequately supplemented. The clinician should review a small animal internal medicine book for recommendations for concurrently managing hypothyroidism in a patient with diabetes mellitus, hypoadrenocorticism, cardiac disease or other significant concurrent illness.

**Conclusion**
Hypothyroidism carries a good prognosis and therapy is inexpensive and relatively safe. However, so many extra thyroidal influences can change T4 values that the misdiagnosis of hypothyroidism may be as common or more common than the actual disease. The clinician should always rely on compatible clinical signs, consider the signalmen as age and breed affect thyroid values and some breeds are predisposed to the development of hypothyroidism. If there is no history of drug administration that can alter thyroid levels or NTI based on history and CBC, serum biochemistry then diagnostic tests to evaluate thyroid function are indicated. If results are equivocal or borderline then retesting in 4 to 6 weeks is a reasonable approach to follow.
Selected References
HYPERADRENOCORTICISM – WHAT SHOULD WE BE DOING?

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Introduction

This article is intended as a brief refresher on current approaches to the diagnosis of canine hyperadrenocorticism (HAC). Some areas of debate and apparent controversy will be highlighted. Spontaneously-occurring canine HAC will be the focus.

Dr Harvey Cushing first described HAC in human beings approximately 80 years ago although he discovered it much earlier. The earliest publication on canine HAC currently listed in Medline was published in 1953. Cushing’s syndrome is synonymous with HAC. The term “Cushing’s disease” is best reserved for cases of pituitary-dependent HAC (PDH).

There is a remarkably large body of peer-reviewed scientific literature dealing with HAC in dogs. A search of Medline for articles on “dogs AND hyperadrenocorticism” on May 18th 2020 resulted in 823 hits, including 22 articles from 2019 and (already) 5 from 2020. This large amount of work has been done because HAC is one of the most prevalent and therefore important endocrine diseases of dogs. Diagnosis and treatment are reasonably complex. Despite a considerable amount of intense study over the last six to seven decades, many cases of HAC in dogs remain challenging to diagnose and some cases are difficult to treat satisfactorily. HAC is considerably less common in cats and people. It is not known why HAC is so common in dogs.

A major difficulty with diagnosis of HAC in dogs is the lack of a single “gold standard” or “reference standard” diagnostic test. As with some other diagnostically-challenging diseases (such as systemic lupus erythematosus), a diagnosis must be based on a combination of compatible clinical findings. In the case of HAC, these findings derive from signalment, history, physical examination, routine clinicopathological testing, diagnostic imaging and adrenal function testing. None of these tests is 100% reliable, the combination must be considered. Compatible history and physical examination findings are particularly important.

It is distinctly possible that the subset of all HAC dogs examined and diagnostically investigated by veterinary dermatologists is subtly different from the subset examined and investigated by internists. Many case series and suggested diagnostic and therapeutic approaches have been published by internists, based on their own clinical caseloads. Therefore caution and perhaps some scepticism should be exercised by veterinary dermatologists if published advice and findings do not seem coherent with their own experiences and clinical findings. In addition, many of the descriptions of canine HAC are decades old. It has been suggested that greater familiarity with the syndrome may have led to earlier diagnoses, perhaps altering the prevalence of some diagnostic features.

Anatomy and pathophysiology of HAC

HAC is a syndrome that arises as a consequence of chronic exposure in the body to excessive cortisol. There are three common kinds of HAC in dogs: 1) a functional pituitary tumor that autonomously secretes excessive ACTH (this is the most common kind, accounting for 80-85%
of spontaneously-occurring cases; PDH); 2) a functionally-active adrenocortical tumor (either an adenoma or a carcinoma, roughly 50:50, 15-20% of spontaneous cases; AT); and 3) iatrogenic HAC as a consequence (usually) of oral or topical glucocorticoid use. Much rarer causes of HAC in dogs include food-induced HAC (presumably due to adrenocortical over-expression of gastric inhibitory polypeptide receptors) and ectopic secretion of ACTH by neuroendocrine tumors or their metastases.

Most dogs with PDH have an adenoma in the pars distalis of the pituitary, fewer have a pars intermedia adenoma or a functional carcinoma. Tumors are described as macrotumours (>10mm in greatest diameter) or microtumours (≤ 10mm). Macrotumors can cause direct, space-occupying, neurological consequences as they become larger. It has been argued that the use of absolute sizes (> or < 10mm), regardless of the size of the patient, is inappropriate given the enormous size variation among dog breeds. An alternative approach that compared tumor height to brain area was devised and first described in 1997 by Kooistra and colleagues but unfortunately it does not seem to have gained widespread favour.

In dogs with PDH, normal feedback inhibition of ACTH secretion by cortisol does not operate properly, although partial responsiveness is present in some cases. Secretion is pulsatile so, consequently, plasma concentrations of ACTH (and therefore cortisol) are highly variable. Although the overall “area under the curve” is increased, a random sample may reveal an ACTH concentration (and cortisol concentration) within the reference range. As a consequence of the chronic ACTH over-exposure, the adrenals both undergo cortisol hyperplasia and enlarge. A variant response in the adrenals is macronodular hyperplasia, which can sometimes be quite strikingly asymmetrical and mistaken for adrenal neoplasia.

In dogs with AT (which is usually unilateral), cortisol secretion is autonomous, independent of pituitary control. The cortisol secreted by the AT causes feedback suppression of hypothalamic corticotropin-releasing hormone (CRH) and ACTH secretion. As a consequence of a lack of ACTH stimulation, the contralateral adrenal gland atrophies considerably over time. It is the size of the smaller adrenal gland that is most diagnostically informative in dogs with AT causing HAC. In some dogs with functional AT, randomly collected serum cortisol concentrations are remarkably low, but it is thought that, nevertheless, the “area under the curve” week-in, week-out, may be abnormally elevated. Some functional ATs have been shown to secrete hormones other than cortisol, yet patients with these tumors showed clinical signs of excessive exposure to glucocorticoid. In a majority of such cases that have been studied, 17-hydroxy-progesterone, rather than cortisol, was the hormone secreted after ACTH administration. The pathophysiology of this form of canine HAC is incompletely understood. A depletion of the enzymes required to complete cortisol synthesis may play a role, with ACTH driving secretion of the precursors instead of cortisol. It is suggested that the secreted precursors may displace cortisol from its binding proteins, increasing the concentration of physiologically-active free cortisol.

Of note, in normal dogs, stress can rapidly affect the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased plasma ACTH and cortisol concentrations, well up into the ranges sometimes used to diagnose HAC. This is why certain samples for diagnosis of HAC (see below) are thought to be best collected from relaxed, calm dogs at home, rather than in the veterinary
hospital. Similarly, dogs with non-adrenal illness often have increased HPA activity, making misdiagnosis of HAC a distinct possibility if dogs with non-adrenal illness are inappropriately tested for HAC before their illness is brought under control.

**Diagnosis—What should we be doing?**

It is remarkable how little some of the most crucial parameters used in the diagnosis of canine HAC have changed over the last four decades. Many reference values and breakpoints have not changed. This is despite the introduction of new therapeutic agents and changes in technology that affect analytical methods used in cortisol assays. There may also have been qualitative changes in our HAC cases (e.g., earlier diagnosis leading to more subtle disease manifestations).

In a 2012 ACVIM consensus statement on the diagnosis of spontaneous canine HAC (Behrend et al. 2013) a key recommendation was that current reference ranges and cut-off values should be re-established. These authors stated that 1) Different assays produce different measured cortisol concentrations; 2) New reference ranges have not in general been generated to accompany the new analytical methods; and 3) The original reference ranges (still in use) were established from studies with various shortcomings. For example, HAC dogs were compared with normal, healthy dogs rather than with control dogs suspected to have HAC but actually suffering from various non-adrenal illnesses. Screening tests were developed in referral settings with high HAC prevalences whereas, nowadays, these tests are commonly used in primary practice settings where HAC prevalences may be much lower. Finally, 4) These assays are being used to test populations that may have a greater proportion of mildly-affected cases, with overall lesser degrees of cortisol excess.

It seems clear that this key recommendation is well-founded and sound. It should be followed. Unfortunately, I can find no clear evidence that this recommendation (published in 2013) has so far been pursued vigorously.

The recommendation about establishing new cut-off values can also be applied usefully to diagnostic imaging, specifically, the assessment of adrenal size using ultrasonography. Normal adrenal size varies with breed, as reported by de Chalus et al in 2013. Reference ranges for a wider variety of breeds would be useful.

*Signalment, history and physical examination findings.* These have been well-described in numerous previous publications and will not be repeated in detail here. Spontaneous HAC affects middle-aged to older dogs, few are younger than 6 years. There is a slight preponderance of females. The reported median age for PDH and AT are similar, about 11.5 years. Most dogs with PDH (~75%) weigh less than 20kg, whereas PDH and AT are roughly equally prevalent in dogs over 20kg.

During history-taking, clinicians should remember to ask questions about topical (e.g., otic, ophthalmic, cutaneous) medications that might contain glucocorticoid as well as oral glucocorticoid medications. Questions should also be asked about anticonvulsant drugs, as some of these can affect adrenal function testing.
The most frequently encountered clinical signs of canine HAC (as reported mainly by internists) are polyphagia, polydipsia / polyuria, panting, abdominal enlargement, muscular weakness and bilaterally symmetrical, usually non-pruritic, truncal alopecia. Less common features are lethargy, calcinosis cutis, bruising, epidermal atrophy, cutaneous hyperpigmentation, comedones, history of urinary incontinence, lethargy, heat intolerance, anoestrus, loss of libido in males, and testicular atrophy. Occasional cases show severe dyspnea (usually due to pulmonary thromboembolism), pseudomyotonia, ruptured ligaments and facial paralysis. Dogs with large pituitary macrotumors may develop dullness, decreased appetite, pacing and aimless wandering, behavioural changes and occasionally stupor or ataxia. Seizures are rare.

Many dogs with HAC have only some or a few of these clinical signs, but at least one will almost invariably be present. If none is present, the clinician is advised carefully to reconsider the tentative diagnosis of HAC.

Another key message from the 2012 ACVIM consensus statement on diagnosis of spontaneous HAC in dogs (Behrend et al. 2013) was that individual clinicians can substantially improve the proportion of their HAC diagnoses that are correct by testing only a carefully chosen population of animals that have one or more relevant clinical findings, as listed above. Careful case selection will substantially improve the accuracy of their diagnostic work by optimising pre-test probability. HAC testing should not be carried out solely on the basis of (for example) certain routine clinicopathological findings. Testing for HAC is expensive and potentially misleading and therefore best reserved for animals with compatible historical and physical examination findings.

The authors of the consensus statement eloquently and concisely made the point that canine HAC is usually a slowly progressive illness, so HAC testing is not mandatory at the time of initial diagnostic suspicion. There is usually no appalling rush. In addition, it is wise to postpone diagnostic efforts for several weeks if the dog has any concurrent illness, especially if it is seriously ill. The stress of any illness can increase cortisol secretion.

If a case is investigated, inappropriately, for HAC on the basis of a single, abnormal clinicopathological finding (for example, an elevated serum alkaline phosphatase, sALP), the unwary clinician is in danger of walking into a “diagnostic quagmire”. The Uncle Remus story about Br’er Rabbit and the Tar-Baby comes to mind. The Tar-Baby was created and put in Br’er Rabbit’s path by Br’er Fox, to catch the rabbit. Br’er Rabbit was angered to receive no reply from the Tar-Baby when he greeted it, so he struck it, becoming stuck. The more he struck the Tar-Baby, the worse he became stuck. In the same way, a first inappropriately selected diagnostic test (a “first strike”) can spawn a second test (in our sALP example, it might be abdominal ultrasonography). Then a third test may be ordered (adrenal function testing or perhaps a liver biopsy). Unintended consequences, patient morbidity and a high cost for the owner can result. Better not to make that first strike without a very good reason.

Routine clinicopathological testing. Common clinicopathological findings in dogs with HAC have been described in detail previously, for example in standard textbooks. A routine hemogram may reveal mature neutrophilia, monocytosis, lymphopenia, eosinopenia, mild thrombocytosis and mild erythrocytosis. There may be an increased number of nucleated red
blood cells. Serum chemistry is likely to reveal elevated sALP, and may reveal elevated alanine aminotransferase (ALT), cholesterol, glucose and triglyceride. The serum bile acids, if measured, may be mildly elevated. Measurement of steroid-induced serum alkaline phosphatase does not provide diagnostic value because it is elevated not just in HAC, but also in a high proportion of animals with non-adrenal illness. The urine specific gravity is usually less than 1.020 (unless glucosuric) and is sometimes hyposthenuric. There may be proteinuria. Urine protein:creatinine ratio, if measured, is usually only mildly elevated. Urine sediment examination may reveal bacteriuria, sometimes in the absence of pyuria. Clinically silent UTIs are common (~40% in females). Therefore, urine bacterial culture (via a cystocentesis sample) is recommended in strongly suspected cases, regardless of sediment findings. None of these alterations is pathognomonic and an individual case is most unlikely to manifest all of them.

Diagnostic imaging. This has been well-reviewed elsewhere (for example, in Bennaim, Shiel and Mooney, 2019).

Bilateral enlargement of the adrenal glands is consistent with PDH, although many affected dogs have normal-sized adrenal glands. Asymmetry of size or shape of the adrenals does not necessarily indicate neoplasia in one or other of the glands. Macronodular hyperplasia (a manifestation of PDH, not AT) can cause marked asymmetry. The \textit{smaller} adrenal gland is the more important one to consider when working to diagnose a functional adrenal tumour. It should typically be very small (or thin, < 0.5cm) in dogs with a contralateral functional AT. Tiny adrenal glands can sometimes be difficult to detect, especially if one is searching for the right adrenal gland, which is usually more difficulty to find. Irregularity of shape, large adrenal size and evidence of vascular invasion are suggestive of malignancy. As a profession, we need to define better the size of \textit{normal} adrenal glands in dogs by establishing reference ranges for different breeds.

Pituitary imaging (CT or MRI) is used if hypophysectomy or irradiation of a pituitary tumour is being contemplated. It can also help in the management of conflicting or discordant results in discriminating adrenal function tests, or if neurological abnormalities are present.

Adrenal function testing. Tests are divided into screening tests and differentiating tests. The most important adrenal function screening tests are the Urine Corticoid:Creatinine Ratio (UCCR), the Low Dose Dexamethasone Suppression Test (LDDST) and the Adrenocorticotropin Hormone (ACTH) stimulation test. Differentiating tests include the endogenous plasma Adrenocorticotropic Hormone assay (eACTH), the High-Dose Dexamethasone Suppression Test (HDDST) and the Oral Dexamethasone Suppression Test (usually combined with the UCCR).

\textit{UCCR} determination is an excellent screening test for HAC in dogs. It provides information about average corticoid production over a longer period than can be achieved by blood sampling. It can be combined with an oral dexamethasone suppression test to aid in the discrimination of PDH from AT. A recommended protocol involves collection by the owner, at home, of two consecutive, free catch urine samples on two consecutive mornings. A morning urine sample is preferred not because of any diurnal variation in corticoid secretion but because a morning sample consists of urine formed over many hours (the overnight period) providing better averaging. It has been advised that collection should not be started until at least 2 days after any
known (or perceived) stressful experience such as a visit to the veterinary practice, so that stress-induced corticoid secretion does not adversely affect the results. Other work has shown that physical examination and blood sampling may not be sufficient to affect UCCR results, but 1.5 days of hospitalisation certainly can be (Galeandro et al., 2014). Starting immediately after the two baseline morning samples have been collected, 3 doses of oral dexamethasone are given (0.1mg/kg every 6-8 hours) and a third urine sample is collected on the next (third) morning. UCCR values for all three samples are determined. The mean of the first two samples is compared to published cut-off values to screen for HAC. If the third sample shows a reduction >50% in UCCR, PDH is diagnosed. If there is a lesser or no reduction, PDH or AT both remain possible and further testing, such as abdominal ultrasonography or eACTH assay can be done. In one study of 160 dogs with HAC (111 with PDH and 49 with AT), the UCCR was suppressed to <50% of baseline in 72% of the dogs with PDH. The remaining 28% of dogs with PDH were resistant to dexamethasone suppression. In the dogs with AT, the maximum extent of suppression was 44% of baseline.

The LDDST is widely used, familiar to most companion animal veterinarians and has changed very little in the last four decades. It is considered by many veterinarians to be the most suitable screening test for canine HAC unless iatrogenic disease is suspected. Reported sensitivity and specificity values vary widely because of the use of differing control groups. In normal dogs, a low dose of dexamethasone is sufficient to inhibit pituitary secretion of ACTH, leading to a lengthy decrease in serum cortisol concentrations (<28nmol/L). A baseline blood sample for serum cortisol is drawn and dexamethasone is administered (0.01-0.015mg/kg iv). Further blood samples for cortisol assay are collected 3-4 and 8 hours later. The test relies upon demonstrating, in HAC dogs, diminished responsiveness of the HPA axis to glucocorticoid negative feedback. Dexamethasone is chosen because it does not interfere in the cortisol assay, as would many other glucocorticoids. In dogs with PDH, there can be variable suppression of serum cortisol concentration, but by 8 hours, the serum cortisol will not be normally suppressed, it will have “escaped”. Dogs with AT do not show any suppression of serum cortisol concentration in response to dexamethasone because their cortisol secretion is independent of ACTH. The LDSST is able, in some cases, to distinguish PDH from AT. Some dogs with PDH show suppression of serum cortisol concentration to below 40nmol/L or 50% of baseline at 4 hours, with “escape” to higher concentrations at 8 hours. Approximately 60% of dogs with PDH show such suppression. Such dogs are suggested to be very much more likely to have PDH than AT. One recent publication has reported the surprising finding that dogs whose serum cortisol is suppressed below 28nmol/L at 3 hours or 8 hours, with the other 3- or 8-hour value being higher, may not have HAC at all and should be evaluated using other tests (Bennaim et al. 2018). Cut-off values used in LDDST should be reviewed and would preferably be determined for each assay method and for each laboratory. 1.3 mg dexamethasone sodium phosphate contains 1mg dexamethasone, clinicians should dose for the actual dexamethasone.

The protocol for the HDDST is identical to that of the LDDST except that 0.1mg/kg dexamethasone is administered intravenously. This is a differentiating test, useful if access to excellent abdominal ultrasonography is problematic. Its value is limited because only about 12% of all dogs with PDH fail to suppress on the LDDST but subsequently do suppress on the HDDST. Nearly 100% of dogs with AT will fail to suppress in the HDDST. The eACTH test is another one that can differentiate PDH for AT. It is not a useful screening test for HAC. Sample
handling is crucially important and low test sensitivity can be problematic unless expensive analysers are available.

The *ACTH stimulation test* is the gold standard for diagnosis of iatrogenic HAC and is used to monitor treatment with trilostane or mitotane. It has less value in the diagnosis of spontaneous HAC. The test takes only 1 to 1.5 hours and can be done at any time of the day. In this test, a relatively high dose of ACTH is used to measure the *maximum* secretory capacity of the adrenal cortices. It is therefore important to use the full, recommended dose of ACTH (5 µg/kg iv). Blood samples for cortisol assay are drawn immediately before and 60-90 minutes after the ACTH injection. Use of compounded forms of ACTH is inadvisable; Cortrosyn, Cosyntropin Injection, or Synacthen should be used. This test performs more reliably for the detection of PDH than for AT. Many adrenocortical tumors do not respond to ACTH by secreting additional cortisol. This produces false negative results if the expectation is that such tumours will respond in that way. Reported sensitivity and specificity values vary widely because of the use of differing control groups and variable inclusion in the studies of adrenocortical tumour-bearing patients. This test has poor sensitivity for the detection of ATs if conventional criteria for interpretation of results are used.

**Summary**

Despite decades of experience in the diagnosis of HAC and some diagnostic tests that have certainly withstood the test of time, considerable diagnostic challenges remain. Multiple diagnostic tests may be required to diagnose cases with HAC, but careful initial focus should be on the basics: signalment, history and physical examination. Careful selection of cases, based on compatible clinical signs rather than on clinicopathological findings alone, will increase diagnostic accuracy. It is not mandatory for more specific HAC testing to begin as soon as the suspicion arises. Delay is sometimes a wise approach, especially if the patient has non-adrenal illness to potentially confuse the picture. It is high time for our profession to re-establish reference values for many of the tests used in investigation of canine HAC, preferably on a per-assay and per-laboratory basis.

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GENERAL DIAGNOSTIC APPROACH TO NODULAR SKIN DISEASES --
IS IT INFECTIOUS, STERILE OR NEOPLASTIC?

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Introduction
The pattern of nodular to diffuse dermal infiltrates encompasses a wide spectrum of infectious
and non-infectious diseases as well as neoplastic conditions. It is important to keep in mind that
the histologic pattern "nodular to diffuse" does not always present clinically as nodules or
masses.

Approach to skin nodules
As clinical presentation equals gross pathology when we deal with skin lesions, providing the
pathologist with adequate detailed clinical information is crucial to get a definitive diagnosis.

Clinical description
Careful description of the actual lesions – location on the body, location as far as dermal versus
subcutaneous, size, number of lesions - should always be considered in association with cytology
as well as histopathology and other additional tests.

Cytology
Smears from fine needle aspirates as well as imprints from excised samples are very helpful to
get a first impression of the composition of the lesions. In addition to evaluation of cytologic
characteristics, these samples can also be used for special stains, immunohistochemistry,
clonality testing and PCR for infectious pathogens.

Imprints from surgical samples: this technique is helpful to get cell samples of neoplastic
populations for evaluation with immunohistochemistry. The surgical sample is bisected and the
cut surface is carefully blotted against a paper towel to remove excessive blood. The cut section
of the tissue is then used to make “imprints” on glass slides. After air-drying, the slides can be
fixed in cold acetone, dried and kept in the refrigerator at -20°C.

Collection of samples for bacterial/fungal culture and or PCR
If there is any concern for an infectious process, collecting additional samples for culture/PCR is
highly recommended. These samples can be kept frozen and submitted later should histology be
suggestive of an infectious process. While samples for culture should be taken after surgical
prepping of the skin surface, biopsies for histopathology should never be jeopardized by cleaning
and scrubbing the skin surface.

Collection of samples for extensive immunohistochemistry
Consult with your pathologist if you are concerned you may be dealing with an unusual round
cell tumor, which may require extensive immunohistochemistry on fresh, snap-frozen tissue
samples.
Categorization of histologic lesions with nodular to diffuse pattern
The low power view of the histologic lesion gives a very important first impression of the lesions and should never be underestimated! It allows to identify: 1) orientation within tissue: random distribution versus targeting specific structures, 2) cellular infiltrate: monomorphic versus pleomorphic and 3) histological evidence of etiology such as infectious pathogens.

Orientation within the tissue
Nodular dermatoses can be subclassified according to orientation of the cellular infiltrate within the tissue.

Periadnexal
The inflammatory nodules are adjacent to or surround the adnexal structures, but lack evidence of infiltration. Keep in mind: marked perivascular mid-dermal dermatitis may look nodular on low power. Examples: Sterile pyogranuloma syndrome

Targeting particular anatomical structures other than follicles
Examples: sebaceous adenitis, angiocentricity of reactive histiocytosis, palisading granuloma focusing on collagen

Random multifocal to diffuse
Examples: bacterial, fungal, algal infections, leishmaniosis, microfilariasis, habronemiasis, foreign body reactions, eosinophilic granuloma, eosinophilic plaque, sarcoidosis, juvenile cellulitis

Type of cellular infiltrate
Keep in mind that rarely is an infiltrate monomorphic, except with neoplastic conditions and even then, there may be a reactive leukocytic population mixed in.
Examples: eosinophils with mast cell tumor or T cell lymphomas, small lymphocytes as an anti-tumor response. Moreover, the type and composition of an inflammatory infiltrate typically changes over time. Despite these facts, identifying the predominant cell population in a pleocellular infiltrate can assist to narrow down the list of differential diagnoses.

Monomorphic infiltrate
The obvious first decision is to differentiate between a neoplastic infiltrate versus a monomorphic inflammatory infiltrate. This can occasionally be challenging.
Examples: reactive lymphocytic inflammation versus small-cell indolent lymphoma (lymphocytosis), plasmacellular pododermatitis versus plasmacytoma, feline progressive histiocytosis versus xanthoma. Clonality testing for rearrangement of T cell receptor rearrangement or immunoglobulin heavy chain rearrangement can assist differentiation between reactive and neoplastic conditions of lymphocytes and plasma cells.

Predominantly neutrophilic infiltrate
The neutrophilic infiltrate peaks by 6 to 8 hours, so it is often very prevalent in acute lesions of various etiologies. Continuous predominance of neutrophils is mostly associated with infectious processes. Special stains assist identification of bacteria. Underlying folliculitis/furunculosis has to be ruled out (absence of entrapped keratin, hair shafts).
Examples of infectious etiology: septicemia, *Neospora*, necrotizing fasciitis (*Streptococcus canis*)
Example of sterile condition: canine sterile neutrophilic dermatitis (Sweet’s-like syndrome)

**Predominantly eosinophilic infiltrate**
This is mostly seen in cats and horses. Admixed there may be lymphocytes and histiocytes. “Eosinophilic mush” are foci of degranulating and degenerative eosinophils surrounded by nodular to diffuse inflammation; this may then become increasingly histiocytic. Some nodules contain brightly eosinophilic collagen bundles, referred to as “flame figures”. The tinctorial change of collagen is likely the result of major basic protein released from degenerate eosinophils and coating the collagen bundles (previously referred to as “collagenolysis”).
Examples of infectious etiology: microfilariasis, feline Herpes virus dermatitis, habronemiasis, pigeon fever (*Corynebacterium pseudotuberculosis*) in horse
Examples of sterile conditions: eosinophilic granuloma, hypersensitivity to Monoject® needles in horses, resolving mastocytosis in horses, eosinophilic plaque, arthropod bite hypersensitivity, canine eosinophilic dermatitis (Well’s-like syndrome), multisystemic eosinophilic epitheliotrophic diseases in horses

**Predominantly histiocytic infiltrate**
Histiocytes (which includes macrophages and dendritic cells) are commonly mixed in with other inflammatory cells, However, they can predominate in certain conditions, both infectious (mostly as functional macrophages) as well as sterile conditions (dendritic cells and/or macrophages). It is important to always consider an infectious etiology that needs to be ruled out with special stains as well as culture.
Examples of infectious etiology: canine lepoid granuloma (novel not yet named mycobacteria (related to *M. tilburgii, M. simiae, M. genavense*), various fungal infections (sporotrichosis, histoplasmosis, blastomycosis, cryptococcosis), leishmaniosis
Examples of sterile conditions: xanthoma (metabolic imbalances: diabetes, hyperlipidemia), calcinosis cutis and calcinosis circumscripta, reactive histiocytosis, uveo-dermatological syndrome in dogs (Vogt-Koyanagi-Harada-like syndrome: cell–mediated hypersensitivity to melanin and melanocytes, endogenous and exogenous foreign bodies, equine sarcoidosis (“naked” granulomas), palisading granuloma (targeting collagen)

**Predominantly lymphocytic/lympho-plasmacellular infiltrate**
Not very common. In most cases this indicates a chronic stage of various inflammatory conditions. It can be seen with persistent antigen stimulation and occasionally exhibit lymph follicle formation. These lesions are erroneously referred to as “pseudolymphomas”.
Example of infectious etiology: borreliosis, mycetoma in horses, ehrlichiosis
Examples of sterile conditions: vaccine reaction, plasmacellular pododermatitis

**Predominantly lympho-histiocytic infiltrate**
As mentioned above, it could reflect a chronic stage of a primary histiocytic process.
Example for a primary lympho-histiocytic lesion: reactive histiocytosis of dogs (cutaneous and systemic form: “bottom -heavy” angiocentric). In the presence of additional more intermediate-sized T cells the consideration is: inflamed T-cell lymphoma, sebaceous adenitis
Granulomatous / pyogranulomatous infiltrate
This is a nodular to diffuse mixed inflammatory infiltrate, which lacks obvious organization and is composed of histiocytes, with various numbers of neutrophils, plasma cells and lymphocytes. This has to be differentiated from discrete well-organized granulomas (see below). Infectious etiology always has to be considered (special stains and cultures) before diagnosing a sterile process.
Examples of infectious etiology: mycobacteriosis, sporotrichosis, botryomycosis, fungal infections, actinomycosis, nocardiosis
Examples of sterile conditions: granulomatous vasculitis, sterile pyogranulomatous dermatitis, nodular panniculitis, juvenile cellulitis

Discrete well-defined granulomas
The composition may be similar to granulomatous/pyogranulomatous, but the inflammation is very organized with neutrophils and histiocytes in the center, surrounded by a ring of lymphocytes and plasma cells. The histiocytic population may also be represented by multinucleated giant cells.
Examples of infectious etiology: periadnexal small granulomas with Demodex mites
Examples of sterile conditions: foreign body granulomas (endogenous: mineral deposits; exogenous: splinters, grass yawn), eosinophilic granulomas within a diffuse eosinophilic inflammation

Selected References
A number of feline flea borne disease agents can be zoonotic. The most risk is from Bartonella spp., Rickettsia felis and Yersinia pestis. Coxiella burnetii DNA has also be amplified from fleas. The purpose of this proceedings is to provide an update on the diagnosis and management of clinical abnormalities associated with Bartonella spp, and Rickettsia spp. infections of cats. Please also see the AAFP reports on feline bartonellosis and zoonoses guidelines www.catvets.com.

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 (Bradbury et al, 2010). None of the six cats administered imidacloprid-moxidectin in that study became infected or febrile. However, not all strains or 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. Lymphadenopathy, endocarditis, myocarditis and hyperglobulinemia are other well documented manifestations of bartonellosis in cats (Whittemore et al, 2012).

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 spp. DNA was amplified from the blood of > 50% of cats housed in an Alabama shelter. 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 (Lappin et al, 2009). 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 spp. 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 (www.dlab.colostate.edu; www.galaxydx.com). 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.

If fever or other clinical signs from bartonellosis is suspected in a cat, administration of doxycycline or a fluoroquinolone is generally effective. Doxycycline at 10 mg/kg, PO, daily for 7 days as the initial therapeutic trial has been recommended by some veterinarians. 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 orbifloxacain

96
have led to rapid resolution of fever in cats with presumed bartonellosis. Azithromycin is now considered contraindicated because of rapid induction of resistance (Biswas et al., 2010). The new veterinary fluoroquinolone, pradofloxacin (Veraflor, Bayer Animal Health) is the least likely to cause resistant strains of *B. henselae* and so may be the preferred quinolone for the treatment of this syndrome (Biswas et al., 2010). Flea control with imidocloprid containing compounds (Advantage Multi and Seresto collar; Bayer Animal Health) has been shown to block transmission of *B. henselae* amongst cats by *Ctenocephalides felis* (Lappin et al., 2013).

The clinical manifestations of bartonellosis in people are more extensive than just cat-scratch disease, peliosis hepatis, bacillary angiomatosis and valvular endocarditis. It is now apparent that immune-competent individuals can develop a number of *Bartonella* species–associated chronic inflammatory syndromes and *Bartonella* spp. infections are an occupational risk for veterinary health care providers (Breitschwerdt et al., 2007; Lantos et al., 2014; Oteo et al., 2017). For example, *Bartonella* spp. infection was commonly confirmed in people with rheumatic symptoms in a Lyme disease–endemic region (Maggi et al., 2012). *Bartonella henselae* may have contributed to the death of 2 veterinarians (Breitschwerdt et al., 2015). Veterinarians or others commonly exposed to cats or fleas that develop chronic inflammatory diseases should have *Bartonella* spp. on the list of differential diagnoses.

**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. *Rickettsia felis* DNA has been amplified from fleas collected from dogs or cats around the world including Australia, France, Israel, New Zealand, Thailand, the United Kingdom and the United States.

Fever, headache, myalgia and macular rash in human beings have been attributed to *R. felis* infection around the world (Angelakis et al., 2016). Rickettsial infection is suspected to 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 (Bayliss et al., 2009). These results prove that cats are sometimes exposed to spotted fever group organisms but further data are needed to determine significance of diseases associations. Because clinical illness in cats has not been documented, optimal treatment is unknown. However, based on results in dogs, doxycycline or a fluoroquinolone would be logical choices.

**Yersinia pestis** (feline plague). *Yersinia pestis* is the facultatively anaerobic gram-negative coccobacillus that causes plague. The organism is maintained in a sylvan life cycle between rodent fleas and infected rodents, including rock squirrels, ground squirrels, and prairie dogs. However, it has been shown that *C. felis* can be a competent vector, but transmission was less efficient than by a rodent flea in one experimental study (Eisen, 2008). Both cats and dogs are susceptible to infection. Antibodies against *Y. pestis* have also been detected in serum of
nondomestic felids. Clinical disease is recognized most frequently from spring through early fall, when rodents and rodent fleas are most active. However, a recent unpublished case in Colorado was diagnosed in December of a mild winter. Most of the cases in human beings and cats in the United States have been documented in Colorado, New Mexico, Arizona, California and Texas. Of the cases of human plague diagnosed from 1977 to 1998, 23 (7.7%) resulted from contact with infected cats (Gage et al, 2000).

Cats and dogs are infected after being bitten by infected rodent fleas, after ingestion of bacteremic rodents or after inhalation of the organism. After ingestion, the organism replicates in the tonsils and pharyngeal lymph nodes, disseminates in the blood, and results in a neutrophilic inflammatory response and abscess formation in infected tissues. The incubation period is 2 to 6 days after a flea bite and 1 to 3 days after ingestion or inhalation of the organism. Outcomes in experimentally infected cats include death (6 of 16 cats; 38%), transient febrile illness with lymphadenopathy (7 of 16 cats; 44%) or inapparent infection (3 of 16 cats; 18%) (Gasper et al, 1993).

Bubonic, septicemic and pneumonic plague can develop in infected human beings, dogs, and cats. Bubonic plague is the most common form of the disease in cats, but individual cats can show clinical signs of all three syndromes. Most infected cats or dogs are allowed outdoors and have a history of hunting. Anorexia, depression, cervical swelling, dyspnea and cough are common presenting complaints; fever is detected in most infected cats. Unilateral or bilateral enlarged tonsils, mandibular lymph nodes and anterior cervical lymph nodes are detected in approximately 50% of infected cats. Cats or dogs with pneumonic plague commonly have respiratory signs and may cough. In a series of 62 suspected dog cases, the most common clinical signs were included fever (100%), lethargy (97%) and anorexia (77%); only 23% of the dogs had lymphadenopathy (Nichols et al, 2013).

Supportive care should be administered as indicated for any bacteremic animal. Cervical lymph node abscesses should be drained and flushed with the clinician wearing gloves, a mask and a gown. Parenteral antibiotics should be administered until anorexia and fever resolve. Optimal antibiotics for treatment of plague in infected cats in the United States are unknown. Streptomycin administered intramuscularly at 5 mg/kg q12h was used historically but is not widely available. Cats treated with gentamicin intramuscularly or intravenously at 2 to 4 mg/kg q12-24h, or enrofloxacin intramuscularly or intravenously at 5 mg/kg q24h, have resolved clinical signs. Chloramphenicol administered orally or intravenously at 15 mg/kg q12h can be used in cats with central nervous system signs. Antibiotics should be administered orally for 21 days after the cat has survived the bacteremic phase; doxycycline at 5 mg/kg q12-24h is an appropriate choice. In one study, 90.9% of cats treated with antibiotics survived, whereas only 23.8% of untreated cats survived (Eidson et al, 1991). In a dog case series, 73% of the 62 suspect cases were treated with antibiotics and 97% of the dogs survived (Nichols et al, 2013). The prognosis is believed to be worse for the pneumonic form of Y. pestis infection. A recent case seen at the authors institution developed a consolidated lung lobe and died after lobectomy.

**Summary.** Fleas on dogs and cats around the world are potential sources of zoonotic agents. When working with pets with heavy flea infestations, veterinarians should wear gloves or wash their hands carefully.
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THE GENETICS OF CANINE ATOPIC DERMATITIS

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Introduction
The clinical manifestation of atopic dermatitis (AD) in dogs and human beings results from a complex interaction between the patient’s genetic characteristics, the clinical phenotype, microbiome and environment. Unlike experimental mouse models, human and canine AD appear to have a complex genetic basis. It is likely that the genomic background influences the variable susceptibility to the disease, clinical signs and severity, and response to treatment between individuals. Knowledge of this could lead to individualised and more effective approaches to diagnosis and treatment.

Methods to identify genetic associations with atopic dermatitis
Early studies relied on heritability studies, which assess the frequency of the condition in family trees. This is useful for studying the inheritance of diseases with single gene defects (e.g., dominant, recessive, sex-linked, etc.). However, it is much less useful in polygenic conditions involving the complex interaction of multiple genetic pathways. While heritability studies can identify predisposed breeds and family groups, more powerful genomic techniques are required to study the genetic background to polygenic disorders. These include candidate-gene association, genome-wide linkage, and genome-wide association studies.

Candidate-gene association studies
It is possible to genotype markers (e.g., single nucleotide polymorphisms [SNPs], insertions, deletions, and repeats) associated with candidate genes of interest. This approach is not limited to families, facilitating inclusion of large numbers of affected individuals and controls. However, candidate-gene studies are hypothesis-dependent and restricted to previously implicated genes. Novel genes may therefore be missed.

Genome-wide linkage studies (GWLS)
GWLS is a family-based approach using affected individuals, their parents and non-affected family members. The inheritance of the disease is compared to the inheritance of microsatellite markers. This technique can evaluate the whole genome, avoiding the limitations of candidate gene approaches. Microsatellite markers have been used to identify chromosomal loci associated AD. However, extensive chromosomal sequencing around these loci is necessary to identify candidate genes. It is also difficult to amass enough affected individuals and unaffected relatives to perform sufficiently powerful GWLS in dogs.

Genome-wide association studies (GWAS)
GWAS is a hypothesis-free way to discover disease-associated SNPs, which avoids the limitations of using candidate-genes while retaining the advantages of the case-control approach. Large microarrays can identify SNPs that are more frequent in affected individuals than controls. These mark regions of the genome that may be involved in the pathogenesis of AD. GWAS can interrogate the entire genome if there are sufficient SNPs and they are evenly distributed but insufficient or uneven coverage can miss associations. In addition, further sequencing and
functional studies are required to confirm that the association is causal. Another weakness is that GWASs are limited to identifying common SNPs with small effects. They cannot identify rare SNPs with large effects, untyped SNPs, and some structural variations (e.g., variable number tandem repeats, insertions, deletions, and duplications). The first GWAS in canine AD used the Illumina Canine SNP20 chip (San Diego, California, USA). This included 22,362 canine SNPs from the CanFam2.0 assembly. Despite this, many genes of interest (e.g., filaggrin) were not included. Since then, many more canine SNPs have been identified and included on microarrays. This has increased the power of GWAS in dogs.

Quantitative RT-PCR
Quantification of mRNA identifies genes that are differentially regulated in affected individuals compared to controls. Microarrays can be used for hypothesis-free assessment of very large numbers of genes on single chips. Gene transcription, however, does not necessarily imply causality; the change may be secondary to the disease process. Despite this, gene expression studies are useful to identify candidate genes and to confirm involvement of genes implicated in other genomic studies. Genome-wide microarrays can produce a hypothesis-free transcriptome of all known genes in any tissue. The cost and ease of these studies is improving rapidly and this approach is likely to be more widely adopted.

Bias and false results
Genomic studies are prone to bias and error that reduce the power of the analyses and affect replication of the results. Accurate phenotyping is critical, as any variation will have a profound impact on determining associations with AD. Other issues include failing to account for population stratification in case control studies. Studies with relatively low numbers of cases are vulnerable to type II (false negative) errors. Type I (false positive) errors can occur following multiple testing unless corrections are used to reduce the false discovery rate. In addition, the effects of non-random mating, mutations, selection, small population effects, genetic drift etc. mean that canine populations may not be in Hardy-Weinberg equilibrium (i.e., allele and genotype frequencies in a population remain in equilibrium).

Heritability in atopic dermatitis
Human AD is familial and strongly associated with atopic traits. The well-recognised breed predispositions for canine AD also support a genetic basis for the condition. However, the lists of predisposed breeds differ widely between studies. This may reflect local breed prevalences in hospital and control populations as well as within-breed variation between different geographical areas. Breeding analyses are not consistent with a simple mode of inheritance, and while experimental colonies of atopic dogs have been established relatively few dogs develop spontaneous clinical disease. The tendency of certain beagle dogs to develop high IgE levels following early allergen sensitization appeared to follow a dominant mode of inheritance. However, this was dependent upon early-life allergen exposure, indicating the critical nature of environmental-genetic interactions.

The mean heritability in British guide dogs (mostly Labrador retrievers, golden retrievers and their crosses) was 0.47, indicating that nearly 50% of the risk of developing AD was determined by an individual’s genotype. As in humans, the risk was greatest when both parents had AD dermatitis, moderate when only one parent was affected and lowest when neither parent had AD.
In a study of Swiss West Highland white terriers (WHWTs) with AD, heritabilities were 0.31 (direct), 0.04 (maternal genome) and 0.03 (maternal environmental).

An early study failed to find a significant association between total IgE levels and canine leucocyte antigen (DLA) haplotypes between healthy and atopic dogs, possibly because most circulating IgE in dog is not allergen specific. However, the combination of the haplotypes DLA-A3 and R15 was found to be present more frequently in atopic versus healthy dogs suggesting that certain immunological phenotypes were predisposed.

**Genetic associations with atopic dermatitis**

Over 30 genes have been implicated in the pathogenesis of human AD, including those that affect innate and adaptive immune responses as well as the development and maintenance of the skin barrier. Similar results from genomic studies in dogs confirm the complexity of this disease.

**Skin barrier function**

Loss-of-function filaggrin mutations are common in human AD and appear to be involved in some but not all affected dog breeds. Filaggrin is a multifunctional skin protein and abnormalities contribute to the skin barrier defects associated with AD. The variable filaggrin abnormalities might be one of the reasons for the variable disease phenotype among breeds. For example, filaggrin gene SNPs were associated with AD in golden retrievers but not in WHWTs. There are also differences in the distribution and type of lesions between breeds. For example, WHWTs have a more generalised and seborrhoeic presentation. It is likely that other genes affect skin barrier development and function in breeds without filaggrin abnormalities. One candidate is plakophilin 2, a protein involved in epidermal adhesion (although one study showing this included German shepherd dogs with low IgA levels in lieu of a clinical diagnosis of AD).

Further studies showed that there were multiple AD-associated genetic variants in specific enhancers involved in gene regulation. Multiple variants appeared to combine in a risk haplotype associated with altered expression of PKP2 and nearby genes resulting in the predisposition of German shepherd dogs to CAD. The cytochrome P450 26B1 gene was associated with AD in WHWTs. This is involved in adipogenesis and retinoic acid pathways, which could result in disruption of normal skin lipid metabolism and skin barrier function. A SNP in RAB3C, which encodes a transporting protein on lipid anchors, was associated with AD in golden retrievers.

**Innate and adaptive immunity**

To date, only the gene for the thymic stromal lymphopoietin (TSLP) receptor appears to be involved in AD in dogs of all studied breeds. TSLP is a keratinocyte-derived cytokine induced by epidermal damage. It initiates TH2-cell responses and stimulates itching. Altered receptor expression or affinity could therefore modulate innate and adaptive inflammatory responses. Other genes involved in inflammatory pathways that have been implicated in canine AD include a protein tyrosine phosphatase that modulates T- and B-cell responses to antigens and PTPN22, which encodes a lymphoid tyrosine phosphatase involved in regulation of T and B cell activation. A GWAS on 35 atopic and 25 non-atopic West Highland white terrier dogs reported significant association with loci on CFA17, CFA6 and CFA9. The loci included candidate genes covering a range of functions including innate and adaptive immunity, skin barrier function, and transcription and regulation. A more recent GWAS of 96 atopic and 87 control WHWTs identified a 2.7 Mb genomic region on CFA3 that included several candidate genes involved in
innate and adaptive immune responses and regulation. A missense variant in the F2R gene was also found in a database of whole genome sequences from 35 dogs of 9 breeds predisposed to AD (although the clinical phenotype was unknown). In other studies, 3 genes have been correlated with CADES-03 scores (S100A8, SAA-1 and PKP2) and four genes with intradermal allergen test results (CMA1, SAA-1, SPINK5 and S100A8); these have been associated with inflammation, T-cell survival and skin barrier function.

Significant associations with high IgE levels have been detected on chromosomes (CFA) 5 and 35 in Labrador retrievers and WHWTs, respectively. However, the clinical relevance of this is uncertain. The Labrador study used house dust and storage mite, fungal, cat and insect allergen specific IgE, IgG1 and IgG4 as phenotypic markers for AD, which may not accurately predict the actual diagnosis. The WHWT study included dogs with high allergen specific IgE levels with or without clinical signs of AD. Moreover, no genes involved in IgE regulation are known to reside in the detected areas on CFA5 and 35.

**Breed and geographical variation**

One large GWAS identified 13 AD-associated SNPs in 659 dogs from 8 breeds (boxer, German shepherd dog, Labrador and golden retriever, shiba inu, pit bull, shih tzu and WHWT) from the USA, UK and Japan. However, only 2 were associated with AD in all eight breeds. The remaining 11 SNPs were associated with AD in individual breeds, suggesting that the atopic genotype is breed specific. Moreover, 5 of the AD-associated SNPs were restricted to breeds from specific geographical locations (e.g., filaggrin with UK Labrador retrievers, and INPPL1 and MS4A2 with Japanese shiba inu). This may explain breed- and region-specific differences in the clinical phenotype of AD.

**Endotyping**

The term endotyping or endotype has been adopted to describe the process of combining data from the genotype, clinical phenotype, transcriptome, metabolome, microbiome and other sources from large numbers of patients with a condition. Bioinformatic analysis is then used to cluster the patients into groups with shared features that may have significance for their diagnosis, treatment and prognosis. In one study in human AD, data was collected from 193 atopic patients and 30 healthy controls. This included the signalment, clinical signs, 147 mediators, total IgE and allergen specific IgE. Principal component analysis revealed 4 clusters with only marginal overlap: high PARC and TH2 cytokines; low RANTES, IFNα and TWEAK; low epithelial cytokines, IFNβ and MIG; and high TSLP, IL1 and TH2 cytokines. These clusters showed differences in clinical signs, severity, concurrent respiratory disease and response to treatment.

**Conclusions**

In summary, canine AD is a familial heritable disease. However, in contrast to conditions with single gene defects that have predictable patterns of inheritance, it is a complex polygenic disorder. New genomic techniques have greatly facilitated identification of AD-associated gene polymorphisms. Despite this, however, the genetic background for canine AD is still far from clear. There has been little correlation between the studies published so far. This variation could be due to the complexity of the genotype, although it is also likely to reflect environmental factors, phenotypic differences between dogs in the studies, breed and regional variation,
different study techniques, mutations with low penetrance, and incomplete genome coverage. Variation can be seen even at the level of the transcription and function of a single associated gene. Finally, studies must be well-designed and sufficiently powerful to avoid bias from non-specific diagnoses as well as breed, regional and environmental variation.

This complexity may explain differences in the clinical phenotype and responses to treatment of affected dogs but will make it difficult to develop simple genetic diagnostic tests for canine AD. Nevertheless, advances in bioinformatics should help link certain genotypes and clinical phenotypes. These techniques will allow identification of target molecules for novel treatments. In addition, we should be able to genotype atopic dogs and relate this to their phenotype. Understanding genotype will enable better targeting of treatment options. For example, some dogs may respond well to skin barrier therapy whereas others would benefit more from allergen specific immunotherapy. We should also be able to identify dogs that may have a poor response, or have an increased risk of adverse effects to certain anti-inflammatory drugs, or both. Finally, it may be possible to discover atopic genotypes in young dogs and manage environmental and other factors to minimise their risk of developing clinical AD.

Genetic data could be used in selective breeding programs to avoid propagating the predisposition to canine AD and reduce the prevalence of the condition. Nevertheless, the complexity and high prevalence of canine AD means that a simple testing, screening and breeding programme to eliminate the condition is unlikely to succeed. Some caution is needed as restricting gene pools too far too rapidly could inadvertently select for other deleterious traits.

Selected References


Canine atopic dermatitis was presented for decades as a type I hypersensitivity disorder characterized by an IgE response and the release of mast cells' mediators. Fast forwarding to our view of atopic dermatitis to 2020, we have realized that the immunopathogenesis of canine atopic dermatitis is much more complex and the IgE response is an epiphenomena in many patients. They are not the cause of the disease per se and histamine is not the most important mediator after all. We have also realized that atopic dermatitis (both in people and in dogs) is not a single disease but a complex clinical syndrome with multiple possible aberrations, which may vary from patient to patient, in dogs from breed to breed and can even vary based on geographical location. All this makes very complex to identify a treatment that would apply to all patients, particularly if the treatment is targeted. This disease is dynamic and different mediators may play a role at different times in the course of the reaction. The disease also appears to evolve over the lifetime of the individual and with the persistence and chronicity of symptoms.

The first step in our understanding of the dynamic nature of the atopic lesions and the various population of lymphocytes playing a role came from studying cytokine expression in atopy patch test reactions. These studies lead to the classic paradigm of Th2 lymphocytes being prominent in the acute phase and Th1 being more relevant in the chronic phase. That artificially simplified view of the complexity of the reactions lead us to largely focus on cytokines like IL-2, which is believed to play a role in chronic phases. Some of the therapeutic strategies like cyclosporine addressed this aspect of the reactions.

As we progressed in our understanding, we realized that the complexity of the lymphocytic response is much larger with more subtypes of lymphocytes and newly discovered cytokines playing a role. We also learned about the role of the epidermis in the development of the immune response during the course of an allergic response. We learned that the epidermis is crucial both for skin barrier function and as modulator of the immune response elicited by exposure to allergens.

It is very clear now that keratinocytes, that were considered inert and a mere physical barrier to the outside world, are instead crucially involved in both orchestrating the immune response and modulation of pruritus. Keratinocytes, for example, are important for the release of Thymic Stromal Lymphopoietin (TSLP) during skin damage. TSLP promotes a Th2 response, and a preferential selection toward an IgE-driven response. Cytokines released by Th2 lymphocytes worsen skin barrier in a variety of ways, thus creating additional release of TSLP and a vicious cycle.

We still do not know the prevalence of primary skin barrier defects in atopic prone dogs prior to development of disease. There is evidence that once the disease has developed, even areas that appear non-lesional from the clinical standpoint, have ultrastructural abnormalities. This can be due to subclinical inflammation or to the presence of primary alterations. More work needs to be done to further elucidate this issue.
Like keratinocytes, dendritic cells of the skin are important orchestrators of inflammation. By producing IL-23, they promote production of IL-22 at the site of inflammation. IL-22 is produced by a variety of cells. Cells that are known to produce IL-22 include Th1, Th22, Th17, neutrophils and macrophages. Its effect in the skin is particularly important on keratinocytes. IL-22 has pro-inflammatory activities and it has been shown to inhibit epidermal differentiation and promotes the induction of pro-inflammatory cytokines. Recent studies in dogs have highlighted the fact that lymphocyte population in the skin of atopic dogs produce IL-22 besides the more well-known Th2 signature cytokines (IL-4, IL-13, IL-31).

Another response to the release of IL-23 is the stimulation of release of IL-17 by a subgroup of lymphocytes called Th17. IL-17 is pro-inflammatory and an inducer of expression of antimicrobial peptides by keratinocytes. This cytokine has been traditionally considered suppressed in atopic dermatitis as atopic patients are very prone to secondary bacterial infections. Contrary to this original belief, there are now reports of the importance of IL-17 in atopic dermatitis in both people and dogs.

Another example of how relevant dendritic cells are in shaping the immune response of atopic patients is that dendritic cells are also responsible for the production of TARC. TARC leads to recruitment and accumulation of CD4+ Th2 cells in the skin. This chemokine has been shown to be overexpressed in canine atopic dermatitis.

From these few examples it is becoming increasing clear that there is an intricate cross-talk between cells in the skin (both keratinocytes and dendritic cells) and the shaping of the immune response, the type of cells that are recruited in the skin, the release of cytokines profiles and the overall effect of this cytokine milieu. It is also increasingly clear that different subgroup of cytokines play a role in different phases of the allergic response, with some being overexpressed throughout. One example is IL-31, which plays an important role in both the acute and the chronic phase. This cytokine was also reported to be present in higher circulating concentrations in atopic dogs. Due to the fact that this cytokine plays a role in both acute and chronic phases of the reaction and that is one of the mediators of itch, IL-31 was a logical target for therapy. Other approaches have been to block the signaling of multiple cytokines. This can be accomplished using Janus Kinase Inhibitors. This approach affects multiple cytokines but only in terms of their ability to activate the signaling pathway and not their actual release. This is why a drug like oclacitinib is not anti-inflammatory per se or at least not similar to glucocorticoids.

Much of the attention when we developed medications to help affected dogs has been on addressing the inflammation with the idea that this will also, in various degrees, address pruritus. We know that histamine does not trigger pruritus in healthy dogs and injection of IL-31 is able to trigger pruritus. This has been reported in several species. An equally important approach to treatment is to focus on mediators of itch and modulators of itch transmission pathways that are not linked to inflammation directly. It is important to realize that keratinocytes and nerve fibers have intricate connections and there is cross talk that modulates the transmission of pruritus. There is increase evidence of a disruption of the neurosensory network activity in atopic dermatitis. This finding is relevant enough to make us consider atopic dermatitis as a neuroimmune disease rather than only an allergic disease. Humans keratinocytes produce neurotrophin-4/5 and the expression is increased in atopic dermatitis, at least in people.
Neurotrophin-4 supports the survival and outgrowth of sensory neurons. Our knowledge of this is in infancy in dogs. In atopic skin, in both dogs and people, there is an increase of intraepidermal nerve fibers and this is responsible for a peripheral sensitization and hyperactivity to stimuli. The increase of intraepidermal nerve fibers is statistically significant even for canine non-lesional atopic skin compared to healthy controls.\textsuperscript{13}

Besides neuropeptides, nerve fibers have receptors for mediators ranging from opioids to endocannabinoids. Decrease of the metabolism of endocannabinoids has been shown to be a successful approach to decrease pruritus in atopic dogs in an experimental setting.\textsuperscript{14} In this study, the product was applied topically to areas challenged with the allergen and a significant decrease of pruritus was noted in dogs treated with the active ingredient when compared to the control group. This randomized placebo controlled study encourages us to consider alternative strategies for the patients that may not respond to currently approved treatments. The modulation of opioid receptor (e.g., stimulation of kappa opioid receptor) is a strategy that is also under consideration to help atopic patients.

In summary, much progress has been done in reference to our understanding of the immune mechanisms of canine atopic dermatitis and this has been reflected in the new treatments that we have available to help affected patients. Importantly, much work is still needed to improve the quality of life of these patients targeting other pathways to modulate pruritus.

References
10. Banovic F, Denley T, Blubaugh A. Dose-dependent pruritogenic and inflammatory effects of


SHOULD ATOPIC DERMATITIS BE REDEFINED AND THE DIAGNOSTIC APPROACH AMENDED?

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University of Edinburgh, Roslin, Midlothian, UK

Introduction
Canine atopic dermatitis (AD) is currently defined as a genetically predisposed inflammatory and pruritic allergic skin disease with characteristic clinical features that is associated most commonly with IgE antibodies to environmental allergens. This definition was first proposed by the American College of Veterinary Dermatology canine AD taskforce in 2001 and was subsequently revised and adopted by the International Task Force on Canine Atopic Dermatitis (now the International Committee on Allergic Diseases in Animals; ICADA). The concept of canine AD as an allergic disease has had a profound impact on diagnostic and therapeutic approaches to the condition.

Our understanding of canine AD has evolved dramatically over the last 20 years, particularly in the fields of epidemiology, genetics, molecular immunology and the microbiome. This has highlighted features that weren’t considered 20 years ago, including heterogeneity in the clinical presentation, variable genetic background, environmental risk factors, skin barrier dysfunction, altered microbiome, immunologic mechanisms driving acute and chronic inflammation and itch, and hypersensitivity to specific allergenic proteins.

The current definition of canine AD encompasses some of its pathogenesis and clinical features. However, it fails to reflect our more recent understanding of the complexity and heterogeneity of the condition. It’s possible that this could perpetuate a ‘one-size-fits-all’ approach to diagnosis and treatment. In particular, there could be an emphasis on IgE-mediated sensitisation to environmental allergens to the exclusion of other triggers and mechanisms.

It is therefore time to review our definition of canine AD light of the advances made in its etiology, pathogenesis, diagnosis and treatment in the last 20 years. This could lead to a better differentiated and more effective approach to diagnosis and therapy.

Is canine AD genetically predisposed?
The evidence for this is covered in another lecture (The Genetics of Canine Atopic Dermatitis). In summary, it is clearly a familial and potentially heritable disease. However, it is a complex polygenic disorder with a variable genotype according to breed and region as well interaction with environmental factors. This complexity will make it difficult to develop simple genetic diagnostic tests and breeding programs for canine AD. However, advances in bioinformatics may help define predisposed genotypes, avoidance strategies, and effective and well-tolerated treatment options.

Clinical phenotypes in canine AD
The standard clinical phenotype in canine AD includes pruritus and erythema, which predominantly affects the face, concave aspect of the ear pinnae, ventrum, axillae, inguinal skin, perineum, interdigital skin, and palmar and plantar aspect of the distal limbs. Conjunctivitis is
also commonly reported, but respiratory signs are rare. Some aspects of AD resemble other skin conditions (and vice versa) and therefore a definitive diagnosis on clinical grounds is impossible.

A set of clinical features (Favrot’s criteria) have been associated with canine AD (see below). Sets of criteria with high specificity are more likely to ensure that a dog has canine AD and minimise false positive diagnoses. However, higher specificity is associated with lower sensitivity – there are more false negatives and some atopic dogs could be wrongly excluded from the diagnosis. The reverse is true for sets of features with higher sensitivity and lower specificity. Interpretation of the actual diagnostic accuracy in terms of the negative and positive predictive values is complicated as this depends on the relative prevalence of AD compared to other pruritic skin diseases in the clinic population. Nevertheless, none of the sensitivity and specificity values are acceptable for making an accurate diagnosis in an individual case. They therefore should only be used to determine inclusion criteria for clinical or epidemiological studies. They could be used to gauge the likelihood of AD in a pruritic dog but this does not preclude performing a thorough history, clinical examination and investigation to eliminate other possible causes for the pruritus.

### Favrot’s criteria

#### Set 1

<table>
<thead>
<tr>
<th>Feature</th>
<th>Reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset &lt;3 years</td>
<td>≥6 criteria:</td>
</tr>
<tr>
<td>Mostly indoor</td>
<td>Sensitivity 85.4%</td>
</tr>
<tr>
<td>Chronic or recurrent yeast infections</td>
<td>Specificity 79.1%</td>
</tr>
<tr>
<td>Affected front feet</td>
<td>≥5 criteria:</td>
</tr>
<tr>
<td>Affected ear pinnae</td>
<td>Sensitivity 58.2%</td>
</tr>
<tr>
<td>Non-affected ear margins</td>
<td>Specificity 88.5%</td>
</tr>
<tr>
<td>Non-affected dorso-lumbar area</td>
<td></td>
</tr>
</tbody>
</table>

#### Set 2

<table>
<thead>
<tr>
<th>Feature</th>
<th>Reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset &lt;3 years</td>
<td>≥6 criteria:</td>
</tr>
<tr>
<td>Mostly indoor</td>
<td>Sensitivity 77.2%</td>
</tr>
<tr>
<td>Non-lesional pruritus at onset</td>
<td>Specificity 83%</td>
</tr>
<tr>
<td>Affected ear pinnae</td>
<td>≥5 criteria:</td>
</tr>
<tr>
<td>Non-affected ear margins</td>
<td>Sensitivity 42%</td>
</tr>
<tr>
<td>Non-affected dorso-lumbar area</td>
<td>Specificity 93.7%</td>
</tr>
</tbody>
</table>

Other complications include breed variation in the clinical presentation of AD (see below), extent of the lesions (localised and generalised), the development of non-specific chronic skin lesions (e.g., alopecia, lichenification and hyperpigmentation), secondary microbial infections, and concurrent ectoparasites etc. It is therefore possible that over-reliance on strict clinical criteria could skew studies towards those breed and individuals that show the best fit.

### Examples of breed variation in lesion distribution in canine AD

<table>
<thead>
<tr>
<th>Breed</th>
<th>Lesion Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalmatian</td>
<td>Lips</td>
</tr>
<tr>
<td>French bulldog</td>
<td>Eyelids, axillae, flexor surfaces</td>
</tr>
<tr>
<td>German shepherd dog</td>
<td>Elbows, hindlimbs, thorax, generalised</td>
</tr>
</tbody>
</table>
Examples of breed variation in the clinical lesions of canine AD

<table>
<thead>
<tr>
<th>Breed</th>
<th>Lesion Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boxer</td>
<td>Urticaria, otitis</td>
</tr>
<tr>
<td>Dalmatian</td>
<td>Decreased pruritus without lesions</td>
</tr>
<tr>
<td>German shepherd dogs</td>
<td>Decreased pruritus without lesions; increased seborrhoea &amp; pyotraumatic dermatitis</td>
</tr>
<tr>
<td>Golden retrievers</td>
<td>Increased pyotraumatic dermatitis</td>
</tr>
<tr>
<td>Labradors</td>
<td>Increased pyotraumatic dermatitis, dry skin, and interdigital furunculosis</td>
</tr>
<tr>
<td>Shar pei</td>
<td>Decreased pruritus without lesions; increased otitis</td>
</tr>
<tr>
<td>West Highland white terrier</td>
<td>Increased <em>Malassezia</em> dermatitis and seborrhoea; decreased conjunctivitis</td>
</tr>
</tbody>
</table>

The role of allergen sensitisation in canine AD

The phrase ‘associated most commonly with IgE antibodies to environmental allergens’ is still widely applied to canine AD. Experimental models of canine AD can be reliably sensitised to environmental allergens and controlled exposure results in acute flares of inflammation that replicate the natural disease. Allergy testing and allergen specific immunotherapy (ASIT) are widely used and can be effective (which is covered in more detail in other lectures in this congress). However, these tests cannot be used to confirm or eliminate AD and should only be used to identify allergens for avoidance and ASIT.

Despite this, a narrow IgE-oriented definition of AD emphasises sensitisation to environmental allergens over other triggers. This can encourage inappropriate testing and therapy to discover the ‘cause’ and ‘cure’ the AD. Most of the data on IgE mediated sensitisation in canine AD has been derived from intradermal allergen (IDAT)/skin prick or allergen-specific IgE serology (ASIS) tests and only a few functional studies using Prausnitz–Küstner (PK), lymphocyte stimulation or basophil degranulation tests have been reported. Furthermore, specific allergens have only been identified in *Dermatophagoides* species house dust mites (group 2, 15 and 18), Japanese cedar (*Cryptomeria japonica*; Cry j 1, 2 & 3) and short ragweed (*Ambrosia artemisiifolia*; Amb a1).

There is little to no regulation of allergens for testing and ASIT in dogs. IDAT test concentrations have been optimised and adjusted, although the recommendations are based on limited data and are largely limited to one source of aqueous allergens. There is marked variation in the protein content between crude allergen extracts and test concentrations may not be universally applicable. Few ASIS tests have published validation data and there are concerns over variable and often poor intra-laboratory and inter-laboratory reproducibility as well as poor correlation with IDATs. Elevated total IgE is predictive of allergic sensitization in human atopic diseases but this isn’t true in dogs, where most circulating IgE is not allergen-specific possibly because of higher endoparasite exposure and/or non-sensitising IgE antibodies. Other causes of uncertainty include cross-reaction between unrelated allergens (e.g., *Toxocara canis* and *Dermatophagoides* species), poor correlation between allergy tests, allergen exposure and
clinical signs, the impact of cross-reacting carbohydrate determinants (CCDs), and non-allergenic activity of certain proteins (e.g., proteases). Finally, given the uncertainty over testing and clinical responses to monovalent allergen preparations (e.g., Allermune® Der p2) it is unclear how ‘specific’ ASIT is and whether this (at least in part) results in non-specific immunomodulation.

Since the original definition of canine AD it has become clear that there are subsets of the condition that don’t involve IgE mediated sensitisation to environmental allergens. These include presumed non-allergic forms with negative IDAT and ASIS (‘atopic-like dermatitis’) and cases where foods appear to be one of or the only trigger (‘food induced atopic dermatitis’; FIAD). More recently, immunological responses to non-environmental antigens have been recognised and it’s possible that dogs develop a condition similar to protein contact allergy in humans.

The role of IgE-mediated sensitization to environmental allergens in canine AD therefore appears to be less certain. This seems to be only one among many triggers of itch and inflammation in affected dogs. It could be more accurate to say that canine AD is a clinical syndrome with multiple and potentially overlapping patterns of allergic sensitisation and includes dogs with no apparent allergies.

**Skin barrier function**
Abnormalities in skin barrier function associated with canine AD include altered lipid metabolism, structural changes in the lipid lamellae and abnormal ceramide profiles, although it isn’t always certain if these changes are primary or secondary (or both). Known and potential effects include increased transepidermal water loss (TEWL), constitutive exposure of Langerhans cell dendrites, immune activation and changes to the cutaneous microbiome.

**Innate and adaptive immune responses**
There has been a huge increase in our understanding of the immunopathogenesis of canine AD. Evidence indicates that cells and mediators associated with the innate immune system are not
simply effectors but have a critical role in initiating and maintaining inflammation in atopic skin though interaction with environmental factors and the microbiome. Mast cells had always been regarded as key players through their position in the skin and IgE-triggered release of potent inflammatory mediators. However, it is now known that keratinocytes are immunologically active and capable of responding to trauma, micro-organisms and other inflammatory stimuli through Toll-like receptor (TLR), TARC, TSLP, S100A8, IFNg, apoptosis and other pathways. This forms an intricate web of interactions between the environment, the innate immune system and the adaptive immunity.

AD was originally associated with a TH2:TH1 imbalance leading to the production of TH2 cytokines (e.g., IL4 and IL13) and IgE production. Subsequent studies using microarrays and other approaches in experimental models and clinical cases of canine AD have revealed a much more complicated and dynamic pattern of lymphocyte activation and cytokine production. Acute lesions are associated with activation of TH2 and TH22 pathways. In particular, there is early expression of the IL31-IL31 receptor pruritus pathway. In contrast, chronic inflammation exhibits more complex and mixed lymphocyte activation and cytokine expression with TH1, TH2, TH17, TH22 and T-regulatory cell responses. There is marked variation in cytokine and other mediator expression between dogs and different stages of the disease, showing that the inflammatory networks in canine AD are complex, variable and evolving.

The microbiome in canine AD
Coagulase-positive staphylococci (e.g., *Staphylococcus pseudintermedius*) and *Malassezia* exacerbate canine AD. These were regarded as secondary infections, but it is now known that they act as conventional allergens, as superantigens inducing polyclonal T-cell activation, and stimulate cutaneous inflammation. This is associated with ‘dysbosis’ rather than ‘infection’ – the microbiome of atopic skin is less diverse than healthy skin and is skewed towards staphylococci and *Malassezia*. Successful treatment is associated with restoration of a normal diverse cutaneous microbiome.

Conclusions
Canine AD is traditionally considered an allergic disease; a genetic susceptibility to TH2 polarised immune responses resulted in the development of allergen specific IgE, which lead mast cell associated pruritus and inflammation on subsequent exposure (the ‘inside-outside’ paradigm). Later studies proposed that an abnormal skin barrier facilitated penetration of allergens, irritants and micro-organisms leading to activation of innate and subsequently adaptive immune responses (the ‘outside-inside’ paradigm). Further insight into the complexities of AD has lead to a more comprehensive theory involving epidermal barrier defects, penetration of allergens, irritants and micro-organisms, poor microbiome diversity, a failure of tolerance and ongoing inflammation (the ‘outside-inside-outside’ paradigm). Despite this, it is still considered a single disease entity, and diagnostic and treatment guidelines rarely address its highly heterogeneous clinical phenotype and pathophysiology. Unnecessary testing and high rates of treatment failure impose a significant financial and emotional burden on owners.
Human atopic eczema (dermatitis) has been defined at its most basic level as a reversible immune-driven dermatosis. Diagnostic criteria are being revised and adapted to better fit clinical and immunological variants of AD. A similarly holistic and inclusive definition of canine AD may now be needed. This should reflect the clinical heterogeneity of the condition between breeds and stage of the disease as well as the complex pathogenesis and array of trigger factors. This will necessitate using the clinical phenotype and biomarkers to develop endotypes and even individual ‘biosignatures’. Avoiding a ‘one disease’ approach and stratifying this highly complex and variable condition into define subgroups will aid individualised medicine with more tailored and cost-effective diagnostic and therapeutic strategies.

**Selected References**


To begin with an obvious statement - the major reason to perform an “allergy test” of any type is NOT to diagnose atopic dermatitis (AD). The reason for testing is to allow the clinician to select allergens for use in allergen-specific immunotherapy, though some may argue that identifying the specific allergens to which a patient is sensitive may also enable the owner to pay closer attention to environmental factors that contribute to allergen exposure. But with recent studies highlighting the great variability in both intradermal and serologic testing, are we deluding ourselves by believing that what we are doing is truly scientific?

Contributions to Variability in Allergy Testing

Timing. In human beings, the results of allergy testing can vary throughout the seasons; there is limited evidence that the same is true for dogs, particularly with serum testing. This is not true with every dog, in every situation, in every region of the world. There are no scientifically established guidelines on when testing should occur.

Serology. Allergen-specific IgE serology (“serum allergy testing”) is increasingly used both by specialists and by general practitioners. When done reliably and used properly, these tests seem to be valuable tools to use in formulating an immunotherapy prescription. These are “IgE Tests” – they measure only the amount of allergen-specific IgE present in the serum and not whether this IgE is functioning to cause an allergic reaction in a pet.

Recent studies have highlighted the extreme variation in veterinary serologic test results from laboratory to laboratory. A prime consideration in the variability of serologic tests is the reagent system used, not only the allergen source but perhaps especially the detection system (e.g., monoclonal antibody-based vs. IgE receptor-based). In human allergology, there exists only a limited number of reagent systems and all are traceable back to WHO reference standards in an attempt to increase reliability. However, even in this situation, different laboratories running the same assay with the same reagents experience variable results over time. Such standards have not been established in veterinary medicine and attempting to do so has proven a rather quixotic task.

Serology is convenient and accessible, but in some cases seems to lack specificity: with this test, a problem may be “false positive” reactions. Some authors call these “clinically insignificant true positives” because the animal may actually have serum IgE against one or more allergens, but without clinical significance. This has, at various times, been attributed to the possible existence of IgE heterogeneity.

Intradermal Testing. Intradermal testing (IDT) is the allergy testing method of choice for many specialists. IDT has the advantage that it is a biological method that in some ways mimics the pathogenetic mechanism of the actual disease. A positive intradermal test requires not only presence of specific IgE antibody, but functional mast cells and microvascular response. It has been considered by some authorities to be more sensitive than serum-based methods. However,
allergen extracts are crude biological materials, differing in allergen content from manufacturer to manufacturer and lot to lot. The syringe full of dust mite extract is likely to vary in composition by practice and over time. Moreover, recent concern has been raised about the appropriateness of the testing dilutions used, which themselves certainly vary by dermatologist. It appears that most dilutions originally used and recommended were selected rather empirically and are still being used despite studies that demonstrate higher allergen concentrations may be more appropriate and are not irritating. This fact alone may be creating enormous variation in patient IDT results, even now.

Implications of this Variability
Both IDT and serologic testing are used to pinpoint the best allergens to include in an allergy shot or allergy drop mixture. But if we can’t do a good job in testing, how can we do a good job with immunotherapy? In fact, studies of response rates to immunotherapy have concluded that the response rates to allergy shots is about the same using either test – roughly 2/3 of dogs benefit from allergy shots, no matter which test is used.

Variability in testing results has been used to advocate for skipping allergy testing entirely. Instead, all pets (regardless of their actual allergic sensitivities) are treated with a standard mixture of allergens that are common in the region. In this case, some animals will clearly be treated with allergens that they are not allergic to, and others may be missing allergens that are important for them. Though this may result in a lower total cost to the owner, the approach is controversial amongst dermatologists. This author’s opinion is that evidence favors use of allergen-specific immunotherapy based on testing, even if imperfect. If owners are to commit to the time and expense of a year or more of allergy shots or drops, the best possible chance of success should be offered, even if it costs a little more to test at the beginning.

Molecular Allergology to the Rescue?
For the great majority of environmental allergens, the specific epitopes to which pets are sensitized have not been determined. In the human allergy world, determining this has been a major effort over the past 10+ years, for several reasons. This information permits standardized dosing, preparation of recombinant allergens, determination of T-cell epitope, use of peptide immunotherapy and other advances. Chief among these has been development of so-called “component testing” or “component-resolved diagnostics” (CRD). Serologic methods using CRD begin not with crude allergen extracts, but with purified recombinant peptides known to be important major allergens. For example, rather than testing for IgE against *Dermatophagoides farinae* crude mite extract, testing is done against Der f 1, Der f 2, Der f 5, and other specific peptides known to be important allergens. This methodology removes the variability inherent with crude extracts. It could possibly lead to more accurate, custom-tailored immunotherapy. It also can enable prediction of clinical risk or management steps. A child may be serology-positive to whole egg; however CRD may reveal that the child can easily tolerate heat-labile egg allergens such that cooked or baked egg products can be consumed safely.

By and large, these molecular methods have not yet been applied in veterinary medicine. With the many uncertainties that exist around current allergy testing practices, there is reason to hope that the future will bring improvements and better reliability.
Skin barrier dysfunction in canine atopic dermatitis has been documented. This can be the result of primary abnormalities of the skin but it can also be secondary to inflammation. Mutations like the ones reported in human patients are yet to be documented as primary causes of skin barrier dysfunction in dogs so many researchers feel that dysfunction in dogs may either be caused by mutations different from the ones that most reported in people (e.g., filaggrin loss of function mutations) or may be due to the effects of inflammation, which can be present in atopic skin, even when it appears clinically non-lesional. Inflammation worsens skin barrier function thus patients with flares have worse skin barrier function. One of the main missing pieces of information in veterinary medicine is studies on skin barrier function in other inflammatory diseases besides atopic dermatitis. These types of studies would help to discriminate whether skin barrier dysfunction is specific for atopic dermatitis or not.

Regardless of the specificity to atopic dermatitis, it is logical to speculate that skin barrier function improves as inflammation and pruritus are controlled. A recently published study that compared the effects of oclacitinib, cyclosporine, prednisone and lokivetmab on skin barrier function parameters like transepidermal water loss (TEWL) and hydration showed that TEWL and clinical scores of dermatitis correlated (the worse the dermatitis, the worse the TEWL) and that some treatments actually improved skin barrier parameters like TEWL and hydration.¹ This was the case for oclacitinib and lokivetmab, which increased hydration and controlled the worsening of TEWL despite repeated allergen challenges in an experimental model of atopic dermatitis. Prednisone worsened hydration and this may be due to the negative effect of glucocorticoids on cutaneous thickness and lipid extrusion.

The upper layers of the epidermis of atopic dogs are deficient in ceramides² and this finding has been the foundation of skin barrier repair strategies that focus on the replenishment of ceramides and overall lipid content of the skin of atopic dogs. Topical application of sphingolipid emulsion can “normalize” the composition of epidermal lipids. These chemical changes correlate with improvement in the ultrastructural abnormalities of the epidermis of atopic dogs.³

Studies on the efficacy of treatments that specifically target skin barrier repair are sometimes challenged by the fact that currently available noninvasive methodologies to assess skin barrier are not always very reliable nor very sensitive. For example, this is the case of measuring TEWL using currently available evaporimeters. For this reason, it is always beneficial to assess the effect on skin barrier in a variety of ways and to correlate the findings with clinical improvement.

Topical application of sphingolipid emulsions aimed at correcting the deficiency of ceramides in the skin of atopic dogs leads to improvement of dermatitis, pruritus and hydration.⁴ This has been reported in several studies, including placebo controlled studies, and has been demonstrated despite allergen challenges in a model of canine atopic dermatitis.⁵ In this recently
published study, an emulsion of sphingolipids and glycosaminoglycans extracts was evaluated in a double blinded, placebo controlled fashion. This formulation is different from previously tested products because the sphingolipid extract contained high amounts of sphingomyelin, a precursor of ceramides. This has been shown to enhance endogenous synthesis of ceramides and to increase lamellar-related structures in vitro. Besides clinical assessment of dermatitis and pruritus, stratum corneum lipidomics analyses were also done at baseline and after 8 weeks of supplementation. Importantly, this approach was used as monotherapy and atopic dogs prone to worsening of diseases were challenged with dust mites several times per week for the duration of the study. Compared to baseline, a significant increase in skin polyunsaturated fatty acids (PUFAs) was seen with the treatment group. In this specific study, the clinical improvement of dermatitis was striking and provides an encouraging finding on how to potentially help patients with a long term and safe strategy.

As atopic dermatitis requires a multimodal approach in the vast majority of patients, some studies that evaluated topical application of lipid based emulsions as monotherapy were found not to be successful in controlling dermatitis and pruritus. This should not be taken as an indication of lack of usefulness of this approach but more an indication that this approach may not be powerful enough as monotherapy for some patients. Indeed this type of strategy should be considered as adjunctive long term approach to nurture the skin and minimize flares.

The main challenge in clinical practice is that in some countries, like the USA, there is a lack of a commercially available spot-on sphingolipid emulsion that practitioners can incorporate as part of their program when helping atopic dogs to be less dependent on rescue medications. There are some shampoos and conditioners with moisturizers and emollients but the only spot-on product available is a combination of essential fatty acids but not a sphingolipid emulsion with ceramides like the ones used in some of the published studies.

Topical application of unsaturated fatty acids and essential oils has been also demonstrated to improve severity of dermatitis and pruritus. Although results for TEWL were varied, the clinical improvement was encouraging. As these therapies are safe and well tolerated, there is no contraindication to incorporating this approach in treatment plans. In a larger study which was randomized, placebo controlled and also used a spot-on formulation containing polyunsaturated fatty acids and essential oils, there was significantly more improvement in CADESI-03 and pruritus scores in the treatment group than in the placebo group. More dogs improved by at least 50% in dermatitis and pruritus scores in the treatment group than in the placebo group (P=0.008 and P=0.070, respectively). No adverse reactions were observed.

Due to the limited number of spot-on products available, skin barrier repair is still not a common component of therapy in clinical practice and most of the emphasis is placed on the rapid control of pruritus rather than a more long-term proactive approach to nurture the skin and minimize future flares. It is hoped that more products will be made available in the future and that clinicians will truly appreciate the importance of skin barrier repair as an important adjunctive therapy for atopic dermatitis.
References
GENODERMATOSES IN DOGS AND CATS

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Excessive scale is the phenotypic manifestation of a failure in skin barrier function. The scale itself is the reparative response and not the actual problem. The practitioner’s goal is to determine what insult has damaged the skin barrier so that an informed treatment can be ensued.

**Cornification** (keratinization) is a complex process by which the epidermal cells undergo differentiation from basal keratinocytes to the highly specialized corneocyte. Basic knowledge of the cornification process will allow the clinician to understand the cause and effect relationship between primary scaling disorders and the phenotypic manifestations.

Often overlooked in biopsy samples, the normal stratum corneum (SC) consists of overlapping layers of bland, lightly-staining, polyhedral, anucleate cells, most of which dissipate during biopsy sampling, cutting and processing. This seemingly inconsequential layer serves as the **key barrier** to protect the body from the external environment. The most important barrier function of the SC is to restrict water movement into and out of the body. Even minor injuries to the corneal layer from tape stripping or applications of solvents will result in increased transepidermal water loss.

When the barrier fails, lipid synthesis is upregulated and the epidermis becomes hyperplastic to deliver more lipid to the SC. Several steps must occur for cornification to proceed normally: (1) bundling of the keratin to establish the corneocyte core, (2) replacement of the cell membrane with a thick cornified envelope, (3) formation of lipid lamellar bilayers and (4) desquamation. A defect in any structural protein or lipid biochemistry will results in upregulation of scale formation to try to seal the barrier.

**Primary versus Secondary Cornification Disorders**

Keep in mind that any damage to the skin barrier will cause a reparative change that is phenotypically unpleasant. The question is whether or not the insult is something that can be repaired or merely managed. It is straightforward to classify these conditions as **primary** or **secondary** disorders of cornification (DOC). A secondary disorder of cornification refers to any insult that drives hyperkeratosis (e.g., ectoparasitism, atopic dermatitis, epitheliotropic lymphoma, metabolic disorders). Primary scaling disorders are typically caused by anomalies in genes that encode structural proteins (e.g., transglumatinases) or enzymes involved in lipid formation or lipid transport. Investigations into the pathogenesis and molecular characterization of primary DOC have far out-paced therapeutic achievements. Secondary causes of scaling need to be ruled out before establishing a diagnosis of a primary cornification disorder. More than 80% of scaling disorders arise from secondary causes.

**What is Seborrhea?**

Seborrhea is a clinical term to describe scaling of any sort: seborrhea sicca (dry), seborrhea oleosa (oily), seborrheic plaque. It technically translates as “flow of sebum”. In the early days of veterinary dermatology, seborrhea was used as a general term for many, if not most, skin
conditions in dogs. The term “primary seborrhea” was used for presumed keratinization disorders long before a plethora of conditions were recognized (e.g., sebaceous adenitis, atopic dermatitis in the West Highland white terrier, ichthyosis, vitamin A responsive dermatosis). Also, the role of *Malassezia* and *Staphylococcus* organisms was not fully investigated until the 1990s. Treatment with imidazoles and newer therapeutics for atopic dermatitis would have likely resolved many of those “primary seborrhea” cases. This outdated term no longer applies to a specific skin disease in dogs.

**Primary Disorders of Cornification**
Classification of a primary disorder of cornification involves the following: 1) clinical features (breed, onset, type and distribution of lesions), 2) histopathologic changes, 3) heritability (recessive, dominant, X-linked), 4) mutation, and 5) loss of target protein or enzyme. Electron microscopy (EM) is a useful tool for studying the pathogenesis of the defect; but only in few instances will EM be diagnostic for a disorder. For the veterinary practitioner, this full work up is generally not needed; supportive clinical features, a minimum dermatologic database (e.g., skin scrapings, tape preparations, dermatophyte culture) and a skin biopsy will often suffice. Primary cornification disorders (e.g., autosomal recessive ichthyosis) are generally nonpruritic and arise in young animals. These disorders have strong breed predilections; however, it should be kept in mind that spontaneous mutations can arise in any animal. For breeding dogs, molecular testing may be needed to further characterize the defect and identify carrier dogs. Commercial and academic laboratories generally offer molecular testing for more common disorders such as Golden retriever ichthyosis.

**Ichthyosis**
In veterinary medicine, the term “ichthyosis” (fish-scale disease) refers to a Mendelian defect in the structural proteins, enzymes or lipids involved in formation of the stratum corneum. On light microscopy, the disorders are characterized as either nonepidermolytic (more common) or epidermolytic (rare).

**Epidermolytic ichthyosis** (epidermolytic hyperkeratosis)
This rare condition in the dog has characteristic histopathologic features (keratinocyte vacuolation and lysis in the upper layers of the epidermis with thickening of the granular layer) that are uniquely correlated with mutations in epidermal keratins. This disorder has been identified in a few dog breeds (Rhodesian ridgeback, Labrador cross), but has been well characterized only in Norfolk terriers. The disorder in the terriers is autosomal recessive and caused by a splice site mutation in the KRT10 gene. The affected dogs have regions of mild pigmented scale with alopecia and roughening of the skin.

**Nonepidermolytic Ichthyosis**
This relatively common form of ichthyosis is seen in several dog breeds and most have an autosomal recessive mode of inheritance. Histologically, affected dogs have marked lamellar orthokeratotic hyperkeratosis with minimal to mild epidermal hyperplasia and dermal inflammation.

Autosomal Recessive Congenital Ichthyosis (ARCI) in American bulldog, golden retriever, Great Dane and King Charles Cavalier Spaniel breeds is due to mutations affecting lipid
metabolism in the epidermis. Abnormalities in the lipid bilayers in the epidermis result in loss of barrier function. American bulldogs have been shown to have excessive transepidermal water loss. The phenotype is the body’s failed attempt to repair the damaged barrier.

The identification of PNPLA1 (patatin-like phospholipase domain-containing protein) mutation as a cause of ichthyosis in golden retrievers led to the discovery of similar mutations in people with ARCI. The affected dogs have generalized large, soft, white to gray adherent scale and ventral hyperpigmentation without alopecia. The phenotype is consistent and easy to recognize. Dogs are typically diagnosed at younger than 1 year of age; however, adult-onset cases can occur. The disease may wax and wane with periodic bouts of exacerbation and remission. Mutational testing has limitations. While all dogs with clinical lesions are homozygous, some dogs can be homozygous but have clinically normal skin. Furthermore, homozygous puppies may be transiently affected; a condition referred to as “milk crust” by breeders. There appear to be other unknown factor(s) that herald the onset of clinical lesions.

A NIPAL-4 mutation account for about 20% of the cases of ARCI in humans, and is the cause of ARCI in American bulldogs. Lesions of ARCI in American bulldogs are apparent within 1 to 2 weeks of birth and stay constant throughout the lifespan of the dog. Young puppies have a disheveled hair coat. The glabrous skin of the abdomen is erythematous with tightly adherent brown scale, which gives the abdominal skin a “wrinkled” appearance. The skin has widely distributed large white scale. Malassezia overgrowth may be severe. The histologic features are similar to those seen in the golden retriever but with mild inflammation due to Malassezia. In adult dogs, this clinical presentation can be easily confused with atopic skin disease.

ARCI in Jack Russell terriers is more severe than the condition seen in golden retrievers and American bulldogs. The defect is due to a mutation in transglutaminase 1 (TG1), which is an enzyme responsible for forming the outer layer of the corneocyte- the cornified envelope. The dogs develop marked adherent “parchment paper”-like scale and Malassezia overgrowth with or without hyperpigmentation. The dogs are likely to have more severe affected barrier defect than the GRs and American bulldogs.

Perhaps one of the most severe forms of ARCI occurs in Great Danes. From birth, the dogs have severe greasy scaling with marked wrinkling of the skin of the face and extremities. All reported puppies in the literature were euthanized due to the severity of clinical lesions. The cause is a mutation in SLC27A4, which encodes for fatty acid transporter 4 and leads to a loss or reduction of the protein. Loss of this transporter can be predicted to adversely affect the organization and lipid content in the SC. The histologic lesions are typical of nonepidermolytic ichthyosis but with unusual accumulation of eosinophilic material (glycosaminoglycan) in hair follicles.

A unique ARCI occurs in Cavalier King Charles spaniels and is recognized at birth by a rough or curly hair coat. The condition is caused by mutation in FAM83H but the function of this gene in relation to cornification has not been determined. The dogs have a syndrome that includes the following features: keratoconjunctivitis from the time the eyelids open, roughened curly pelage, scaling with abdominal hyperpigmentation, footpad hyperkeratosis, nail dystrophy, and periodic nail sloughing.
Selected References
METABOLIC AND PARANEOPLASTIC SKIN DISORDERS IN DOGS AND CATS

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No published proceedings for this presentation—this page left blank for taking notes
LASERS IN VETERINARY DERMATOLOGY
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Introduction
Light is electromagnetic energy consisting of photons (or packets) of energy that travel in a straight line, in a waveform, from its source until something acts on it to alter its direction. The first LASER (light amplification by stimulated emission of radiation) was created by Maiman in 1960.\(^1\) Surgical lasers were introduced for use in medicine through the 1970’s and first reported for veterinary use in the late 1980’s. Since then many different media have been used to create different lasers, each with their own characteristics and target tissue types.

How Laser Light is Generated
A laser consists of a laser medium enclosed in a chamber between two parallel mirrors. One mirror is a total reflector and the other partially reflects and partially transmits (therefore allows the light to pass through and out). An electrical source is applied to the chamber exciting the atoms in the laser medium and raising them to a higher state. This state is unstable and the medium preferentially returns to its ground state by releasing energy as a photon of light (with a specific wavelength). These photons are initially released in all directions. Those moving to the sides of the chamber are absorbed, but those moving to the mirrors are reflected back through the chamber where they interact with the medium causing more atoms to be raised to an excited state with subsequent photon release. This generates an increasingly intense beam of photons of the same wavelength (monochromatic), travelling parallel and in phase with each other. The partially reflecting mirror at one end of the lasing chamber then allows release of the photons in a cohesive beam.\(^2,3\)

Types of Lasers and Tissue Interaction
Generally, lasers are named after the material used in the lasing media and this also determines the specific wavelength of the energy produced. The lasing media may be gas, liquid, solid crystal or diode and over 40 types have been developed for medical and industrial use.

A chromophore is a molecule within tissue that absorbs laser light. Each wavelength is absorbed maximally by a specific chromophore within the target tissue and this allows lasers to be developed for very specific targets and different uses. Examples of different lasers are seen in the following table

<table>
<thead>
<tr>
<th>Laser</th>
<th>Wavelength (nm)</th>
<th>Absorption chromophore</th>
<th>Absorption depth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Argon</td>
<td>488, 514</td>
<td>Haemoglobin, melanin</td>
<td>&lt; 1 µm</td>
</tr>
<tr>
<td>Diode</td>
<td>630-980</td>
<td>Melanin, water (range)</td>
<td>&gt; 5 mm</td>
</tr>
<tr>
<td>Nd:YAG</td>
<td>1064</td>
<td>Melanin</td>
<td>&gt; 5 mm</td>
</tr>
<tr>
<td>Er:YAG</td>
<td>2940</td>
<td>Water</td>
<td>&lt;0.1 mm</td>
</tr>
<tr>
<td>CO₂</td>
<td>10 600</td>
<td>Water</td>
<td>&lt;0.1 mm</td>
</tr>
</tbody>
</table>

When the laser beam strikes tissue it may be reflected, transmitted, scattered or absorbed, and may result in a variety of effects including photothermal, photochemical or photoacoustic reactions.
Photochemical reactions occur when the energy of the laser creates a chemical change in the tissue through excitation and rearrangement of molecular bonds. An application of this is the use of a photosensitizing agent that is light activated and lesion localizing in photodynamic therapy. The laser energy is absorbed by the photosensitizing agent resulting in the production of reactive oxygen species and free radicals, and therefore causing destruction of the tissue containing the photosensitizer. Red light is commonly used and does not result in any thermal effects in the target tissue.

Photoacoustic or photomechanical effects occur when a sudden (nanosecond or less) pulse of extremely high energy density is generated. Steam formation causes expansion, creating a mechanical shockwave in the tissue that travels at supersonic speed and can cause mechanical rupture. An example of this is the use of dye laser for lithotripsy (destruction of ureteral calculi).

Photothermal effects refer to the absorption of laser energy by tissue with the conversion of energy to heat. The effect that is seen is dependent on the energy density of the beam, the wavelength of the laser, and the absorption coefficient of the tissue. This is the principle mechanism induced by lasers used in soft tissue surgery for dermatology.\textsuperscript{3,5,6}

When laser energy is absorbed and converted to heat the tissue temperature increases. At 42\textdegree – 60\textdegree C, hyperthermia may contract, constrict or destroy blood vessels. As the temperature increases to 60\textdegree to 100\textdegree C, coagulation occurs, collagen contracts, proteins are denatured and irreversible damage occurs. Superheating the tissue above 100\textdegree C causes vaporization where the intracellular water is vaporized, and desiccated cellular contents are released as a plume of smoke along with approximately 99\% of the heat generated. Continued lasing results in carbonization (char formation) or blackening of the tissue. Char changes the optical properties of the tissue, resulting in a marked increase in the scatter, reflection and energy absorption of any wavelength. If further lasing of the char occurs, the laser energy is absorbed (without the possibility of energy release via vaporization) and thermal energy accumulates in the tissue in the form of radiant heat. The heat is conducted to the surrounding tissue leading to hyperthermia and collateral tissue damage, which may result in delayed healing, increased risk of infection or dehiscence.\textsuperscript{3,5,6} If insufficient power is applied to the tissue, the thermal energy will diffuse into the surrounding tissue before an ablation threshold is reached leading to shallower area of vaporization and wider area of collateral thermal injury.

There is no single laser wavelength that will perform every kind of surgery optimally as each wavelength has its optimal chromophore or target molecule in the tissue. Generally, for most procedures in veterinary dermatology, the CO\textsubscript{2} laser is the laser of choice.

**CO\textsubscript{2} Laser**

The CO\textsubscript{2} laser’s wavelength of 10 600nm means its chromophore is water and the absorption coefficient of water at this wavelength is very high, whilst the scattering and reflectance coefficients are very low. This means that the water will absorb the energy, resulting in vaporization of the cell instead of coagulation. The depth of collateral damage is only ~ 100 \(\mu\)m allowing precise incision and ablation.
The precise effect of the laser on tissue is further affected by factors that can be altered by the surgeon, including power setting on the laser, spot size of the laser beam and speed of movement of the beam across the tissue. Energy is the measure of work (Joules) and power is the rate at which the work is done and is measured in Watts (Joules/sec). The size of the area in which a fixed amount of energy is delivered will determine the magnitude of work accomplished in that area. The smaller the spot size, the greater the energy concentration delivered to that spot. This is known as the Power density (W/cm²) and for each increment that the diameter (of the laser spot) changes by a factor of two the power density changes by a factor of four. For example, the 10W power setting delivered in a spot size of 0.8 mm has a power density of 2000 W/cm²; decreasing the spot size to 0.4 mm increases this to 8000 W/cm² and decreasing to 0.2 mm delivers 32 000 W/cm². So even though the power output setting remains the same the energy density delivered varies tremendously along with the potential impact on the tissue. The power density needed to vaporize tissue is 1200 – 1500 W/cm².

The beam generated in a CO₂ laser has a lens that focuses the beam into a focal point (Figure 1). Moving the hand piece so that the tissue surface is at the focal length (focused) or further away (defocused), allows different power densities to be delivered without needing to alter the power setting on the laser. Some lasers also have the ability to adjust the tip size on the hand piece, as well as, focusing and defocusing the beam.

![Figure 1](image.png)

The distance from the handpiece tip to the focal point also varies depending on the laser type. Articulated arm lasers (where the beam is delivered via a series of 7 mirrors) have a relatively long focal point (~ 3 cm) with narrow angles of convergence and divergence. Therefore, very large movements are needed to defocus the beam. Waveguide fibers (where the beam is delivered by a hollow flexible guide) have hand pieces with a focal point of ~ 2 mm, do not require an aiming beam and have wide angles of divergence. Therefore, small changes to the distance from laser tip to tissue surface cause marked differences in the power density (Figure 1). By moving the tip of the laser hand piece closer to or further away from the tissue it is possible to keep the power output the same but change from cutting to ablating to coagulating the tissue (to provide hemostasis). If precise cutting is required with minimal collateral thermal damage, the power density should far exceed the vaporization threshold, whilst it cannot be exceeded if coagulation is the aim.

The timing of the beam generated when the footswitch is depressed can also be adjusted and there are 4 main settings: single pulse, repeat pulse, continuous wave and superpulse. The single
pulse mode generates a single pulse of laser energy each time the footswitch is activated and is useful for marking out the lines of incision before tension is applied to the skin. The repeat pulse mode cycles between on and off for as long as the footswitch is depressed, and both the pulse duration (msec) and frequency (cycles/sec) can be adjusted. This is used for very precise ablation of tissue in sensitive areas (e.g., third eyelid or laser myringotomy). In continuous mode the beam generation lasts as long as the footswitch is activated and this is the most frequently used mode.

In superpulse mode, the beam is delivered in very short pulses (typically microseconds) at very high peak power, so that the ablation threshold is reached before the tissue thermal relaxation time (the time it takes for the energy to be absorbed and start to diffuse into surrounding tissue). This allows very high power outputs to be applied resulting in efficient cutting without heat diffusion or an increase in the collateral thermal damage. Superpulse is therefore a very useful mode for char free incisions and ablation but has decreased hemostasis at the ablation margins.

**Diode Laser**

These lasers operate in the near infrared spectrum (625 – 1400nm) and the photons produced are weakly absorbed by soft tissue, including water. Their advantage is that the beam produced can be transmitted through quartz fibers allowing their use through endoscopic equipment. As the beam is not absorbed by water they can also be used in immersed operating fields (e.g., ears filled with water during a flush procedure). Diode lasers can be used in 2 modes: contact or noncontact. In noncontact mode, the energy is highly scattered in the soft tissue resulting in coagulation, which may extend for several millimeters surrounding the tip of the laser fiber, allowing use for nonfocused deep tissue coagulation. In contact mode, the tip of the fiber is “carbonized”, by coating the surface with carbon before use. The carbon absorbs the laser energy causing the tip of the fiber to heat (900 - 1500ºC) and the hot tip then acts as a nonlaser, thermal ablation device. While they have their applications, these lasers must be used with care as the thermal damage may extend well beyond the point of contact and continue after the beam is terminated due to heat accumulation and thermal relaxation.

**Clinical Applications**

There are many advantages of the CO₂ laser for use in veterinary dermatology surgery. The wavelength is highly absorbed by water allowing very accurate photothermal ablation of tissue with minimal collateral damage and very good hemostasis of the soft tissue capillaries. This provides a dry surgical field in even highly vascular areas. The CO₂ laser caps nerve endings resulting in less post-surgical pain and animals seem to return to normal behavior faster than with conventional surgery. As the lymphatics are sealed as the tissue is cut there also appears to be less post-operative swelling. While the CO₂ laser can be used in any situation a scalpel can be used, it has many distinct advantages, some of which will be detailed in a selection of the following conditions.

*Nodular sebaceous hyperplasia.* This is a common non-neoplastic tumor, seen more commonly in some breeds (poodle, cocker spaniels, beagles, schnauzer). While usually present as a solitary lesion, large numbers may be present in some individuals. The laser has a particular advantage in these cases as it is possible to remove large numbers quickly by either excising through the base
or vaporizing the mass without need for clipping or preparation. Most are left to heal through second intent, although larger ones may be sutured or closed with surgical glue.

**Follicular tumors.** The site is clipped and prepped and the laser used to make a small incision. After that the power can be reduced as the tumor is dissected from the surrounding fat. This is relatively easy as these tumors have clearly identifiable wall.

**Actinic (solar) keratosis.** These lesions are seen on non-pigmented skin due to excessive UV-light exposure. On cats, they are commonly seen on the pinna (particularly the lightly haired areas) and the nasal planum, and may be a precursor to squamous cell carcinoma. On dogs, they may be seen on the non-pigmented and lightly haired skin of the nasal planum, muzzle and periorcular skin, but are also common on the non-pigmented areas on the ventrum of chronic sunbathers. The lesions may range from single, small well circumscribed foci of hyperkeratosis to hundreds of lesions that tend to coalesce. Individual lesions can be ablated with a slightly larger spot size (0.8 mm) but more widespread superficial resurfacing can be accomplished with a 3 mm paintbrush tip.

**Bowenoid in situ carcinoma.** This is a precancerous lesion of cats associated with a papilloma virus infection. The lesion appears as multifocal crusted plaques in any location (i.e., are not just associated with UV-light exposure). The area needs to be clipped, as often the affected skin extends well beyond the area of crust formation and is recognised by hyperpigmented, slightly infiltrated plaques. Both the crusted areas and the areas of hyperpigmentation need to be removed, using a 3mm tip in superpulse mode to minimize collateral thermal damage. It is only necessary to ablate to the basement membrane and even quite extensive areas may be ablated in a single session. Post-operative care consists of pain relief for 7 days, cleaning if serum oozing is present and an antibiotic cream (silver sulphadiazine) in deeper areas of ablation.

**Pigmented viral plaques.** This is seen in dogs with a strong predilection for pugs, miniature schnauzers and French bulldogs although they have also been seen in other breeds (golden retrievers, Boston terriers). They can range from single lesions to large numbers forming large coalescing plaques, more commonly on the ventrum. The lesions are removed in a similar fashion to Bowens of cats.

**Squamous cell carcinoma.** Occur most commonly in areas of UV-light exposure (nasal planum and pinna in cats, planum, muzzle and nonpigmented skin on the ventrum of sunbathing dogs). In early lesions with no visible extension into surrounding tissue, the tumor may be excised using a 0.25mm tip or ablated using a 1.4 or 3mm tips. Complete nasal planectomy in cats is very well tolerated and appears to cause significantly less pain then if accomplished using cold steel dissection, as the cats are often eating within a few hours of recovery. A planectomy is only viable if the tumor has not progressed and spread beyond the borders of the cartilage.

**Apocrine cytomatosis.** This is seen in cats and appears as blue/grey fluid filled cysts in the ear canals and on the concave pinna. The numbers may vary from individual lesions to hundreds that tend to coalesce occluding the canals and causing chronic otitis externa. The laser is used to first ablate the epithelial covering allowing the fluid to escape. Once this has been blotted away, the medial aspect of the cyst can be ablated using a higher power setting with wider tip.
**Proliferative hyperplasia of the auditory meatus.** In some dogs with chronic otitis externa, marked proliferative hyperplasia of the connective tissue, apocrine glands, sebaceous glands or combinations of the three occur. This results in an exophytic proliferation that completely fills the vertical canal and concave aspect of the pinna causing occlusion of the canal opening and vertical canal. In some individuals, there may also be proliferation of the horizontal canal but in others it is relatively unaffected. The laser is ideally suited to remove this tissue, using a combination of excision and debulking with a 0.4 or 0.8 mm tip and then sculpting and vaporizing the remaining excess tissue using a 3mm tip. Post-operative pain relief and topical antimicrobials are provided until healing occurs (generally fully healed by 3 weeks). This procedure removes all of the proliferative tissue and recreates a relatively normal vertical ear canal allowing access to and treatment of any remaining disease in the horizontal canal and avoiding the need for a total ear canal ablation and bulla osteotomy.

Other conditions in which laser therapy may be used with marked benefits over conventional cold steel surgery include oral viral papilloma removal, oral calcinosis circumscripta, gingival hyperplasia, plasma cell pododermatitis and canine interdigital follicular cysts (see Duclos 2008 for a complete description of this procedure).

**References**

What’s the future of therapeutics in 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 veterinary practice. It is therefore important that we 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 produce some kind of immunologic response. Familiar examples include vaccinations for infectious diseases 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 or coronavirus patients was used to treat sick victims. An increasingly common example is the use of monoclonal antibodies or other substances such as “fusion proteins” therapeutically.

An Introduction to 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 arose from many different clones of antibody-producing cells, each clone producing antibodies that bound to different regions of the substance, and were therefore called “polyclonal”. If instead, we immunize a mouse, harvest antibody-producing spleen cells from the mouse and fuse individual cells with an immortalized antibody-producing lymphoid cell line, we end up with cells that are all a single clone and 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 these monoclonal antibodies are mouse IgG, as they are produced by mouse cells. 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 nearly exactly like a human (or dog) antibody so that it will not be recognized as foreign. There are various scientific
ways of engineering the mouse proteins 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 a standard code, for example [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 another standard code - [xi] if a chimeric antibody; [zu] for a humanized antibody; [u] as a fully human antibody; or [vet] for an antibody of veterinary species. 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.** 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, tumor cell, cell of the immune system, cytokine or a cytokine receptor. Familiar examples on the market currently include adalimumab (anti-TNF-alpha, for human psoriasis, rheumatoid arthritis, and Crohn’s disease), omalizumab (anti-IgE, for human allergic diseases) or rituximab (anti-CD20, a lymphocyte marker, therefore used to treat human lymphoma) and many others. An interesting human mAb is dupilumab, which binds to and blocks the receptor for IL4/IL13. It has shown great promise in treating refractory human atopic dermatitis. At least ten other mAbs are currently in development for human atopic dermatitis. Unfortunately, all of these mAbs would be ineffective in dogs or cats, as they are engineered specifically for human beings (“humanized”).

**Use in Veterinary Medicine.** 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 have seen the appearance of therapeutic mAbs in our own clinics. Development of mAbs against canine IL-31 (lokivetmab) has unarguably led to stunning success in some dogs with atopic dermatitis. Antibodies against the cytokine Nerve Growth Factor are under development for treating chronic pain in dogs and cats. A molecule that inhibits IL4/IL13 action is also reportedly under study. We have the strong advantage of evaluating results in human dermatologic disease with an eye towards pets. Once a promising target for human dermatopathy is identified and an effective mAb constructed, it is a reasonably straightforward task to translate that knowledge to dogs, cats or horses.

**Active Immunization.** Veterinary medicine has taken a further leap forward in biologic therapies that has not yet been evaluated in people – active immunization. The principle here is to intentionally induce autoantibody against a “self” antigen by immunizing with cleverly-constructed molecules that force the immune system to break its normal immunologic tolerance. For example, equine IL-5 has been incorporated into a novel vaccine based upon cucumber mosaic virus. When injected into horses, the animals develop titers of antibodies against their own IL-5. In essence, the horse generates “its own mAb” thus obviating the need for frequent injections of a potentially expensive mAb preparation. Horses with insect bite hypersensitivity
have shown clinical relief upon injection of this experimental product. The same principle has been used in experimental canine atopic disease, using a canine IL-31 “vaccine.” Even forcing a cat to make antibodies against its own Fel d 1 allergen is possible. This not only renders the cat less likely to cause allergic responses in cat-sensitive owners, but provides ethicists with a wealth of issues to debate.

**Is There a Downside?**

What could go wrong with biological therapies? What about safety? 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 acids, 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 will probably 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. In the specific case of lokivetmab, data from the manufacturer indicates about 3% of dogs will develop “anti-drug antibody” which might render subsequent injections ineffective. 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 very 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.
BIOFILMS AND THEIR IMPLICATIONS FOR CLINICAL PRACTICE IN VETERINARY DERMATOLOGY

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When we speak of “bacteria” and “bacterial infections,” the image that comes to mind is free-living, metabolically-active organisms that rapidly reproduce and create pathogenic effects in the host. However, microbes can exist in several distinct forms, not only the “planktonic” or free-living form we commonly envision but also as “sessile” organisms within biofilms. Sessile bacteria within biofilms are radically different than their planktonic forms in many ways and have unique properties that can contribute to disease.

What Are Biofilms?
Biofilm formation might be thought of as “bacterial hibernation.” When a growing, planktonic bacterial cell in an infection encounters a surface (epithelial or artificial), it often adheres to the surface, first somewhat tentatively then later with strong and irreversible attachment. Microcolonies then form, and at a certain point, quorum-sensing mechanisms begin to slow the metabolism of the bacteria and induce them to produce extracellular polymeric substances (EPS) consisting of a slimy mixture of DNA, proteins, and polysaccharides. The EPS forms a thick, sticky shield around the colony that physically protects it from the environment, including from the effects of antimicrobials. The biofilm-protected organisms exist in a kind of stasis. Upon later stimulation, some bacteria emerge from their resting state and transition to planktonic form again, fully able to grow and reproduce and re-initiate or prolong the infection as the cycle continues.

Contrary to usual thinking, most organisms in nature are not in planktonic form, but rather exist within biofilms. Biofilms are the “natural state” of bacteria on rocks, in rivers, and in soil, and the same is true for organisms on the skin and mucosae. The hibernating bacteria are not necessarily bad, because normal commensal “healthy” bacteria within biofilms – ubiquitous on healthy skin - can serve as a continuing reservoir to compete with pathogens, to stimulate keratinocytes to produce antimicrobial peptides, and to otherwise function in a protective capacity for the host.

But what about when things go wrong? What happens when pathogen-containing biofilms form on orthopedic implants, intravenous catheters, chronic wounds or even within the middle ear? Or when these pathogens, protected by biofilm and subjected to subinhibitory concentrations of antibiotics, are then able to become resistant to the antimicrobials and newly release resistant planktonic organisms into tissue? It is clear that these situations can have dire consequences for the host, even to the extent of sepsis and death.

Organisms that Produce Biofilms
A very large number of bacterial genera are capable of producing biofilms, but in veterinary dermatology the ones of most importance are probably the staphylococci and Pseudomonas aeruginosa. Results of studies vary, but it is safe to say “most” strains of these organisms are capable of producing biofilm.
Biofilm production is not limited to bacteria. *Malassezia* yeast can also do so, and under the right circumstances, even dermatophytes. Sadly, disease states relative to fungal biofilms have received little study to date even in people – and perhaps predictably, not at all in species of veterinary importance. What about that Westie with recurrent yeast otitis, or the Basset hound with chronic *Malassezia* dermatitis? Or the Persian cat with intractable *Microsporum canis* infection? Studies on the possible role of fungal biofilms in skin disease might yield strong insights into treatment.

**Skin Disease and Biofilms – What is Their Impact?**

Given the above, it is not difficult to imagine a wide variety of animal skin diseases wherein biofilms might play a factor. Unfortunately, virtually nothing is known about this directly in dogs or cats. We can, however, provide some excellent examples in human beings that almost certainly would apply to our own dermatology patients.

*Nonhealing Wounds.* This is the classical example. Numerous studies have demonstrated that traditional culture methods may grossly under-report the bacterial strains present in a wound (as compared with molecular methods) and that organisms can persist in biofilms, impervious to the action of antibiotics.

*Atopic Dermatitis.* It seems clear that nonpathogenic strains of staphylococcus are resident flora in healthy skin, including strains hibernating in biofilms. Atopic dermatitis (AD) is now strongly associated with cutaneous “dysbiosis” with a shift in the skin microbiome towards *Staphylococcus aureus* (man) or *S. pseudintermedius* (dogs). In human beings, *S. aureus* not only releases enterotoxins with superantigen properties, active in perpetuating the IgE/allergic inflammatory response, but keratinocytes exposed to *S. aureus* biofilms rapidly undergo apoptosis (which does not occur with planktonic *S. aureus*). The damaged, apoptotic keratinocytes can then promote an inflammatory, pruritic response through TLR-3-mediated secretion of thymic stromal lymphopoietin – not to mention the dead cells creating possible interference with epidermal barrier function. Restoration of the cutaneous microbiome has become a therapeutic focus in some recent studies of AD.

*Difficult Otitis.* There is no question that bacterial biofilms play a role in acute otitis media of children. In these patients, traditional cultures are negative, while both bacterial DNA and mRNA – often of *Hemophilus* – can be detected by molecular methods from the effusion fluid. Studies of *P. aeruginosa* isolates from canine ear effusions show that most bacterial strains are exuberant biofilm-producers. Logically, one could easily envision this as a reason for apparent treatment failure and/or recurrence – though this has not been demonstrated in clinical patients.

*Mysteriously Antibiotic-Responsive Skin.* In people, some recent attention has been focused on skin diseases that are not thought to be caused by infection, yet are inexplicably responsive to antibiotics. Often, this effect is rightly attributed to some immunomodulatory effect of the drug, independent of its microbicidal action. But there is another explanation: biofilms are present, containing either difficult-to-culture or completely sessile organisms, ergo the negative culture in the face of disease related to bacteria or their metabolites. Such has been the situation with acne vulgaris and *Propionibacterium acnes* in people, among others.
Treatment Implications
Should we focus more attention on how to remove, disrupt, or interfere with formation of biofilms in patients with chronic skin or ear infections? Perhaps this is why certain formulations of topical products seems to work better for some patients, even when they contain identical “active ingredients.”

Disrupting Biofilm. Currently in the veterinary dermatologic global market, two ingredients are being promoted for their potential ability to disrupt biofilms and thereby provide superior efficacy: silver ion (as topical microparticulate silver, with silver slowly released once applied to the skin or ear) and N-acetylcysteine (in ear irrigation or treatment solutions, or even administered orally). Both of these ingredients have good in vitro data to support their antimicrobial and anti-biofilm properties, but controlled clinical trials demonstrating convincing efficacy are lacking.

One does not have to spend much time reading the literature about biofilms to conclude that this is a subject that is extremely under-studied, with great potential impact on treatment of everything from staphylococcal pyoderma to allergy to ear infections. Further investigation into the role of biofilms in veterinary skin disease is clearly warranted.
MANAGEMENT OF RESISTANT STAPHYLOCOCCAL INFECTIONS IN DOGS –
THE AUSTRALIAN PERSPECTIVE

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Management of resistant staphylococcal infections in dogs in Australia is not greatly different in principle from management elsewhere in the world. What differences the Australian situation may have with other parts of the world can relate to:

- antibiotic susceptibility and possibly virulence differences in the strains predominant in the different geographic regions of Australia
- drug availability and prescribing regulations
- availability and type(s) of bacterial culture and sensitivity testing
- predominant breeds and breed susceptibility
- cultural differences in client expectations, compliance and finances

A Brief History of MRSP in Australia

*Staphylococcus pseudintermedius* is part of the normal skin flora in dogs and is typically carried in the mouth, nostrils and perineal regions. Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) is defined by the presence of the mecA gene. The mecA gene is a chromosomal gene that encodes production of a modified penicillin-binding protein (PBP2a) that has a low affinity for β-lactam antibiotics leading to clinical resistance to all of this drug family. The mecA gene is located on a mobile element called the ‘staphylococcal chromosomal cassette’ (SCCmec) and can be transferred between different staphylococcal species. The SCCmec can also carry resistance genes to other antibiotics.

MRSP was first reported in 1999 in North America and subsequently in Europe, Asia and the middle East.¹ It was first reported in Australia by Siak et al with cases dating from 2011.² Canine MRSP pyoderma was first diagnosed in Melbourne at the Animal Skin and Ear Specialists (ASES) in May 2013. It was very likely present long before this time, but the incorrect testing protocol being used at the time (using cefoxatin, not oxacillin, antibiotic discs for testing) meant that false negative results were produced. It was over 12 months before most commercial laboratories in Australia changed testing protocols. Because of this MRSP may have been present in Australia as early as 2000 but was unable to be diagnosed because of incorrect commercial laboratory protocols.

An Australia wide survey of 888 isolates from 22 laboratories from January 2013 to January 2014 found 11.8% to be MRSP.³ Of these, 19 stain types (STs) were identified amongst the MRSP isolates with five dominant (>five isolates) STs present: ST71, ST497, ST316, ST496 and ST45. The distribution of strains varied markedly with geography. Ten STs were novel and were assigned new sequence types. ST497 was only isolated from Melbourne and thought to have likely evolved locally. 52% of all isolates were extensively drug resistant (XDR, 1-2 sensitive antibiotic groups), 38% multi-drug resistant (>2 antibiotic group resistances) and 10% low level resistant (LLR) MRSP.⁴ At ASES in 2019, 26.7% of methicillin-resistant *Staphylococcus* (MRS) isolates were XDR, 40.0% MDR and 33.3% LLR.
Management Principles
The principles of management of MRS pyoderma in the dog are no different to management of pyoderma with methicillin-sensitive Staphylococcus pseudintermedius (MSSP):

- use the right drug(s)
- use at the right dose
- use for the right duration
- remove impediments to response
- attempt and control the underlying disease process that triggered the infection to minimise the risk of relapse, if not done so already.

The route of treatment and duration of therapy is typically based around the depth of bacterial pyoderma present:

- surface infections (bacterial overgrowth; limited to the surface of the epidermis)
- superficial infections (bacterial folliculitis; infection extending to the hair follicles)
- deep infections (bacterial furunculosis or cellulitis; infection extending to the dermis, typically associated with ruptured hair follicles).

Assuming efficacy of therapeutics, all other things being equal, an MRSP infection should clear at the same rate as an equivalent MSSP infection. On the other hand, there are distinct differences in epidemiology and hospital management of MRSP pyoderma cases compared with MSSP.

A Short Note on Hospital Management of the MRSP case. While more extensive detail is beyond the scope of these notes, we routinely use personal protective equipment (gloves / shoe covers / gowns) for all MRSP cases at ASES and see them in a dedicated MRSP consult room to try and minimise lateral spread within the clinic. The room is cleaned following every MRSP appointment with 60-90% alcohol spray on the benches and equipment and 1:125 F10 (Chemical Essentials Pty Ltd) on the floor following sweeping.

Right drugs: Topical antiseptics with chlorhexidine or bleach are effective in a majority of cases of MRSP when applied with adequate frequency and where practical and appropriate should be used in all cases. These can be effective as a sole therapy in cases of surface or superficial pyoderma and should be used as an adjunct to antibiotic therapy.

Oral antibiotic use is strongly recommended in most cases of MRSP deep pyoderma (because of inadequate penetration by topical antiseptics). It can also be appropriate for superficial pyoderma but given the poor blood supply to the stratum corneum in particular, is not the best pharmacokinetic choice for surface infections. Selection of antibiotics should only be made based on in vitro sensitivity testing (typically disc sensitivity testing in the Australian context). Given the extent and variability of antibiotic resistance in Australian MRSP strains, trying to guess clinical antibiotic efficacy is fraught with failure. There are several caveats however:

- some laboratories only test for a very limited number of antibiotics. For XDR and some MDR MRSP isolates, this may leave the clinician with no antibiotic options. To minimize this risk, the clinician should speak with all of their available laboratories to ensure samples go to the lab with the widest variety of antibiotics available for testing (assuming equivalent veterinary relevant protocols are being used). Failing this, the
clinician should speak with the laboratory to discuss further testing strategies (e.g., sending the sample to a reference laboratory) should all tested antibiotics test resistant. These discussions are best had long before any samples are sent.

- **zone of inhibition (ZoI) breakpoints extrapolated from human *Staphylococcus* sensitivity testing may be incorrect for dogs.** Relatively recent studies looking at doxycycline and minocycline and *S. pseudintermedius* in dogs have highlighted this problem.\(^6\)\(^7\) If human ZoI breakpoints are used, then resistance for both these antibiotics is underestimated. This may be more of an issue where only human laboratories are available to the clinician. At the time of writing, CLSI (Clinical and Laboratory Standards Institute) standards have incorporated the new ZoI doxycycline breakpoints for dogs but these are not yet published for minocycline. If a sensitive result is returned for minocycline, ZoI data should be requested. If the ZoI is <23mm, resistance should be assumed.

- **availability of commercial mupirocin testing is rare in Australia (and is not calibrated for *S. pseudintermedius* in dogs).** While high-level resistance to mupirocin has been reported in *S. pseudintermedius*, multiple studies show it to be infrequent. Nonetheless, the lack of testing available makes this a less desirable option if other antibiotic options are available.

- **availability of commercial florfenicol testing is rare in Australia (and is not calibrated for *S. pseudintermedius* in dogs).** Florfenicol is available for topical use in dogs as a long-acting ear treatment (Osurnia, Elanco Animal Health). In *Staphylococcus* infections, the presence of chloramphenicol is commonly mediated by inactivation by chloramphenicol acetyltransferase (CAT) enzymes, but this is not predictive for florfenicol resistance, because structural differences render florfenicol resistant to CAT enzymes. In *Staphylococcus* organisms, two additional resistance genes have been identified that mediate resistance to both chloramphenicol and, in most cases, florfenicol: *cfr* and *fexA*. One German study examining the prevalence of florfenicol resistance in chloramphenicol resistant *Staphylococcus* from multiple animal species found only 11/188 resistant to florfenicol as well as chloramphenicol; none of these were from dogs (n=44) and none were *S. pseudintermedius*.\(^8\) While this may appear to make florfenicol a potentially attractive option for the treatment of MRSP otitis, the reporting of a case of acquisition of the *fexA* and *cfr* genes in *S. pseudintermedius* during florfenicol treatment of canine pyoderma would suggest caution should be exercised in its use.\(^9\)

Lastly, the significance of drug interactions should not be ignored. Rifampicin in particular, while often recommended to be used in conjunction with other antibiotics to reduce the risk of development of resistance, can interact with many other medications (including multiple antibiotics [see table] as well as prednisolone and cyclosporin), typically speeding their metabolism and reducing their bioavailability and blood concentrations, reducing efficacy.

*Right dose:* Pharmacokinetic / pharmacodynamic studies have been done for many of the antibiotics for treatment of *S. pseudintermedius* in the dog. The resulting doses (see table) are not always the same as manufacturer recommended doses and the clinician needs to be aware of these differences to maximise the benefits of therapy and keeping the course as short as possible, while minimising risks associated with therapy. The impact of food at the time of dosing on both
antibiotic pharmacokinetics and adverse reactions should also not be ignored. Check patient
weights carefully prior to therapy!

**Right duration:** Most surface and superficial infections will be successfully managed in 2 to 3
weeks, though deep infections may take 4 to 6 weeks or longer. Every case of bacterial
pyoderma should have a recheck at the end of therapy to reassess the infection and whether it has
responded appropriately. Cytology is critical at this stage. In this way, antibiotics are not used
longer or shorter than is necessary.

If there is complete clinical response of the infection and negative cytology, then the clinician
should look at historic relapse rates and consider workup and management of underlying
triggers, or topical maintenance therapies as required to prevent relapses.

If there is steady improvement and no or few bacteria remaining, then another week or two of
therapy followed by another recheck is indicated. If improvement stalls and significant numbers
of cocci are still evident on cytology and compliance has been good, then repeat the culture to see
if there has been a change in sensitivities.

**Remove impediments to response (and assessment):** The goal of therapy must be to clear the
infection as quickly as possible and this is more important when antibiotics with an increased
risk of adverse reactions are being used. To achieve this, any impediments to a rapid response
should be removed.

Allergies are a common cause for underlying infection and a variety of antipruritics are
commonly employed. However, there is some evidence to suggest that concurrent use of these
drugs when trying to clear infection, specifically for superficial and deep infections, may be
counterproductive to rapid infection resolution.

- Prednisolone has been reported in one study of 216 dogs to delay resolution of pyoderma
  at ‘anti-inflammatory’ doses, presumably by inhibition of cytokine production and
  inflammatory responses essential for fighting infections.\(^\text{10}\)
- In mouse models, suppression of interleukins 4, 6 and 13, all targets of oclacitinib
  (Apoquel, Zoetis), have been found important in susceptibility to subcutaneous infection
  or sepsis with *S. aureus*.\(^\text{11}\)
- The conclusion of a pediatric study of severe atopic eczema with *S. aureus* skin
  infections was that anti-infective treatment administered before cyclosporin is likely to be
decisive for the long-term therapeutic effect. The same study showed no significant
  impact where there was only *S. aureus* colonisation.\(^\text{12}\)
- Low concentrations of IL-31 stimulate expression of antimicrobial peptides at
  concentrations below that which interfere with the physical skin barrier. Complete
  suppression of IL-31 signalling may be undesirable.\(^\text{13}\)

In addition to the above, there is no evidence that the author could find to show that concurrent
use of these medications has no impact on speed or success of anti-infective treatments in
superficial and deep pyodermas. For these reasons, **where it is clinically possible, anti-
inflammatory therapies should be withdrawn for the duration of antimicrobial therapy.**
Concurrent use of lokivetmab (Cytopoint, Zoetis) to control allergic pruritus is less likely to be problematic but it’s efficacy in the face of significant infection may be compromised.

If use of glucocorticoids, cyclosporin and oclacitinib are given for the duration of antimicrobial therapy, strong consideration should be given to their cessation, if possible, for the seven days prior to the recheck. In this way, they do not interfere with assessment of clinical lesions secondary to anti-microbial therapy, which otherwise may be suppressed if the anti-inflammatories are continued up to the recheck.

Control of the underlying disease process: Where underlying disease processes can be corrected during antimicrobial therapy (typically parasitic diseases and endocrine diseases), this should be done (though levothyroxine has been noted as a possible risk factor for adverse reactions with rifampicin).14

Allergy diagnostics and management typically can wait until after resolution of the infection, because while allergies are a common cause for infection relapse and can cause primary pruritus and inflammation, they are not a cause for failure of infection resolution. Avoidance of identified or suspected contact allergens, where relevant, may be useful in improving clinical outcomes sooner. Commencement of elimination diet trials at the start of antibiotic therapy may not be wise as it may confound the cause of inappetence should it occur as an adverse reaction to an antibiotic.

Clinical Tips on Commonly Used Topical Antiseptics for MRSP Treatment

Chlorhexidine: 53% of cases of canine superficial pyodermas responded to sole therapy with twice weekly 2% miconazole, 2% chlorhexidine shampoo (Malaseb, Dermcare Pty Ltd) and 30 to 62.5% were cured with various 3% chlorhexidine shampoos.15 More frequent bathing (up to daily) has been anecdotally reported but irritant side effects may prevent more frequent bathing in some dogs. Dilution of shampoo prior to application, and thorough rinsing and drying, should decrease this risk.

To reduce the frequency of bathing without reducing antisepsis, the author commonly uses aqueous 2% chlorhexidine in a spray for large regions, sponged on for dogs that do not like sprays and using makeup pads for smaller skin folds. 2-3% chlorhexidine containing mousse (Douxo Pyo Mousse, Ceva) and antiseptic wipes (Douxo Pyo Pads, Ceva; Biohex Wipes, Betbiotek Inc.) can also be used to treat localized infections. Antiseptics however do not penetrate the skin and are not typically adequate for sole therapy of a deep pyoderma / furunculosis.

The efficacy of topical antiseptic therapy efficacy as a preventative for infection relapse has not been studied in dogs. However the author will often use

- at least weekly Malaseb or 3% chlorhexidine shampoo (Pyohex, Dermcare Pty Ltd), with 3% chlorhexidine leave-on conditioner (Pyohex Medicated Conditioner, Dermcare Pty Ltd) afterwards
- up to six times weekly 2-3% aqueous chlorhexidine OR
- up to three times weekly 1:2 diluted 3% chlorhexidine conditioner (Pyohex Medicated Conditioner, Dermcare Pty Ltd) applied to dry or damp skin
as symptomatic management of relapsing pyoderma, for symptomatic management long term or while definitive treatment for underlying disease is being instituted.

Other Antiseptics: While this author prefers chlorhexidine for most cases of MRSP, there are occasional cases where 3% aqueous chlorhexidine is required (though this can occasionally cause irritation) or when chlorhexidine fails. In these cases, considerations include 0.2% diluted bleach, accelerated hydrogen peroxide (Oxybac hand wash, SC Johnson Professional), and polihexanide/EDTA (Otoflush, Dermcare Pty Ltd) depending on the size of the skin region affected.

<table>
<thead>
<tr>
<th>Antiseptic</th>
<th>Concentration</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorhexidine</td>
<td>2% (20mg/ml)</td>
<td>Concentrate can be made up as a spray and applied once daily. Do not use Pyohex Medicated Conditioner (Dermcare Pty Ltd) any more often than every second day as maceration of the skin may occur with daily use.</td>
</tr>
<tr>
<td>Sodium hypochlorite (bleach)</td>
<td>0.2% (2mg/ml)</td>
<td>Add bleach to water not water to bleach to minimise fumes. Use in a well ventilated region. Rarely causes irritant reactions. Inactivated by organic debris.</td>
</tr>
</tbody>
</table>

Clinical Tips on Oral Antibiotics Potentially Useful for MRSP Treatment

Fluoroquinolones: Fluoroquinolones are a known risk factor for development of MRSA in people and their routine use should be avoided. In Australia, while MRSP isolates have been strongly associated with resistance to fluoroquinolones, some are susceptible. Given their relative safety, pharmacokinetics and the fact that they are likely unaffected by co-administration with rifampicin, fluoroquinolones are an appealing choice for MRSP infection treatment when required. However,

- To minimise the subsequent development of resistance, the ‘mutant prevention’ doses should be used.
- If sensitivity testing shows resistance to any fluoroquinolone, then assume resistance to all.
- Pradofloxacin has no additional benefit in treatment of MRSP infection over other veterinary fluoroquinolones.
- The extremely variable absorption of ciprofloxacin in the dog means it cannot be recommended.

Clindamycin: In Australia, MRSP isolates were strongly associated with resistance to clindamycin. Inducible resistance to clindamycin is an uncommon problem, but if there is erythromycin resistance with clindamycin sensitivity, then assume inducible resistance and avoid use of clindamycin. Clindamycin bioavailability is good with or without food, but the hydrochloride salt can cause esophageal scarring if not completely swallowed, so dosing with food is preferred.

Doxycycline: Do not use if resistance to tetracycline is reported as MRSP resistant to tetracycline will NOT respond to doxycycline. Doxycycline is available in both monohydrate and
hydrochloride salts. While dosing with food is recommended to minimise nausea (absorption is unaffected), the monohydrate salt is preferred to minimise any risk of esophagitis associated with incomplete swallowing.

**Minocycline:** Minocycline is still effective in some (around 4-28% based on ASES data) cases where MRSP is resistant to both doxycycline and tetracycline. The presence of the tetK resistance gene in the absence of tetM will give this picture. It should not be considered unless the lab indicates sensitivity (see limitations on this above). Bioavailability is better when dosed fasted, but nausea can be an occasional problem and the availability of the antibiotic only as the hydrochloride salt raises the risk of esophagitis with incomplete swallowing.

**Fusidic Acid:** Fusidic acid is available as 250mg tablets but is very expensive. Pharmacokinetic studies of fusidic acid orally in dogs are limited to a single study, demonstrating inferior pharmacokinetics compared to human beings but better than other tested animals (which did not include cats). The dose rate suggested is partly anecdotal but appears to be efficacious in the author’s experience. In the human literature, resistance has been reported to develop if given as monotherapy, but in XDR MRSP cases sensitive to only fusidic acid and rifampicin, and given the negative interaction reported between these two drugs, the author has used it periodically as a monotherapy (in combination with topical antiseptics) without resistance development to date. Interestingly, in Melbourne, fusidic acid resistance rates seem to be higher in MSSP isolates compared with MRSP isolates. While nausea has been an occasional adverse reaction, because of the issues with potential resistance and poorly understood bioavailability, this drug should not be used unless there are no other alternatives.

**Trimethoprim Sulphonamide:** While an antibiotic with very favorable bioavailability, there are concerns regarding idiosyncratic and immune-mediated adverse effects in some dogs, especially with prolonged therapy, including keratoconjunctivitis sicca, hepatopathy, hypersensitivity and skin eruptions. Its use in Doberman pinschers should be avoided as they are at greater risk for possible side effects. Drug-induced hypothyroidism is possible especially at higher doses. If prolonged (>7 day) therapy is anticipated, baseline Schirmer’s tear testing is recommended, with periodic re-evaluation and owner monitoring for ocular discharge. Its efficacy in severe purulent infections is questionable because the mechanism of action of sulfonamides is inhibited by an excess of p-aminobenzoic acid (PABA), such as found in tissue exudates.

**Chloramphenicol:** This is an antibiotic used rarely by the author because of the narrow safety margin, human safety risks, potential drug interactions, requirement for it to be compounded in Australia and poor compliance because of the three times daily dosing requirement and often large capsules required. High doses more often produce toxicity in dogs and gastrointestinal (GI) disturbances are common. In large dogs especially, chloramphenicol may lead to reversible hind limb ataxia. Rare irreversible idiosyncratic aplastic anemia has been described only in people and clinicians should caution pet owners about handling the medications, and to ensure that accidental exposure does not occur at home. Chloramphenicol will inhibit the metabolism of multiple drugs, including opiates, barbiturates, propofol, phenytoin and salicylates.

**Rifampicin:** There is good evidence that this drug, when used at the pharmacokinetically ideal 5-6mg/kg once daily given fasted, is effective as a monotherapy with relatively few side effects.
when dosed for 3 weeks or less,\textsuperscript{24, 25} despite the potential for a wide range of problems. Compounding is often necessary to achieve this dose.

Rifampicin is a strong inducer of drug metabolizing enzymes leading to increased clearance and lower serum concentrations of many other co-administered drugs, including most antibiotics that may otherwise be effective for MRSP. In people, four weeks is required for full recovery of the rifampin effect after discontinuation.

Adverse effects include liver injury (potentially severe) and GI disturbance. Two separate canine studies found liver enzyme disturbances more likely at 19-28 days or later suggesting that, while weekly blood testing has been recommended, short courses of rifampicin are less likely to cause liver perturbations.\textsuperscript{24, 26} Adverse reactions have been reported more likely with co-administration of TMS, doxycycline and levothyroxine sodium.\textsuperscript{26} 1000IU vitamin E per 20kg q24h may help reduce the chance of hepatotoxicity.\textsuperscript{27} \textbf{However, any dog on rifampicin with appetite reduction should cease the drug until liver enzymes have been assessed.} Rifampin also has an unpalatable taste, may produce a discoloration (orange-red color) to the urine, tears and sclera, and may have adverse effects on sperm quality in breeding dogs.

Rapid development of resistance has been reported in human beings and dogs.\textsuperscript{28} While concurrent use of other antibiotics has been recommended to reduce the risk of resistance, there is little evidence to support that recommendation, especially in light of pharmacokinetic studies demonstrating unfavorable interactions with most antibiotics. The author has experienced rapid resistance development as an infrequent clinical issue within 1 to 2 weeks of dosing. Aggressive use of concurrent topical antiseptics is recommended to minimize this risk.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MRS Dose Rate</th>
<th>Dosing with Food / Fasted</th>
<th>Interaction with Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin</td>
<td>8-11mg/kg twice daily &gt;11mg/kg once daily</td>
<td>With food</td>
<td>↓ concentration*</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>20mg/kg once daily</td>
<td>Either</td>
<td>NR</td>
</tr>
<tr>
<td>Marbofloxacin</td>
<td>5mg/kg once daily</td>
<td>Either</td>
<td>NR</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>5mg/kg twice daily</td>
<td>With food</td>
<td>↓ concentration*, ↑ risk adverse reactions \textsuperscript{26}</td>
</tr>
<tr>
<td>Minocycline</td>
<td>5mg/kg twice daily</td>
<td>Fasted</td>
<td>NR</td>
</tr>
<tr>
<td>Fusidic Acid</td>
<td>25-40mg/kg twice daily (ideally double dose first day)</td>
<td>Fasted</td>
<td>↓ concentration*, ↑ rifampicin concentration*</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>5-6mg/kg once daily</td>
<td>Fasted</td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-Sulphonamide</td>
<td>15-25mg/kg twice daily</td>
<td>Either</td>
<td>↓ concentration*, ↑ risk adverse reactions \textsuperscript{26}</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50mg/kg three times daily</td>
<td>Either</td>
<td>↓ concentration*</td>
</tr>
</tbody>
</table>

* Based on human studies

NR None reported
References

A PRACTITIONER’S APPROACH TO IMMUNE-MEDIATED SKIN DISEASES

Peter B. Hill
University of Adelaide School of Animal and Veterinary Sciences, Adelaide, South Australia

Many immune-mediated and autoimmune skin diseases have been reported in the veterinary literature. As these cases are relatively uncommon (or even rare), they are often difficult to recognize in the general practice setting. Treatment of these conditions can also require regimes that are less familiar to general practitioners. For these reasons, the diagnosis and management of these conditions can be challenging.

Table 1 shows an alphabetical list of the most widely recognised immune-mediated and autoimmune skin diseases. While such a list can be useful for looking up conditions in a textbook, it is of no value in helping a clinician to arrive at a diagnosis and it can be overwhelming.

Table 1.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Condition</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alopecia areata</td>
<td>Lichenoid dermatoses</td>
<td>Pseudopelade</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>Linear IgA disease</td>
<td>Pyoderma gangrenosum</td>
</tr>
<tr>
<td>Auricular chondritis</td>
<td>Lupoid onychodystrophy</td>
<td>Sarcoïdosis</td>
</tr>
<tr>
<td>Bullous pemphigoid</td>
<td>Lupus (cutaneous/DLE)</td>
<td>Sebaceous adenitis</td>
</tr>
<tr>
<td>Bullous SLE</td>
<td>Lupus (systemic)</td>
<td>Sterile eosinophilic pustulosis</td>
</tr>
<tr>
<td>Cryoglobulinaemia</td>
<td>Lupus (vesicular)</td>
<td>Sterile granuloma/pyogranuloma syndrome</td>
</tr>
<tr>
<td>Degenerative mucinotic mural folliculitis</td>
<td>Lupus (exfoliative)</td>
<td></td>
</tr>
<tr>
<td>Dermatomyositis</td>
<td>Lymphocytosis/pseudolymphoma</td>
<td>Sterile neutrophilic dermatitis</td>
</tr>
<tr>
<td>Drug eruption</td>
<td>Mixed autoimmune subepidermal</td>
<td>Sterile nodular panniculitis</td>
</tr>
<tr>
<td>Eosinophilic mucinotic mural folliculitis</td>
<td>Myospherulosis / Spherulocytosis</td>
<td>Sterile pustular erythroderma</td>
</tr>
<tr>
<td>Epidermolysis bullosa (acquisita)</td>
<td>Mucous membrane pemphigoid</td>
<td>Stevens Johnson syndrome</td>
</tr>
<tr>
<td>Erythema multiforme</td>
<td>Pemphigus erythematous</td>
<td>Subcorneal pustular dermatosis</td>
</tr>
<tr>
<td>Follicular mucinosis</td>
<td>Pemphigus foliaceus</td>
<td></td>
</tr>
<tr>
<td>Granulomatous mural folliculitis</td>
<td>Pemphigus vegetans</td>
<td></td>
</tr>
<tr>
<td>Histiocytosis</td>
<td>Paraneoplastic pemphigus</td>
<td></td>
</tr>
<tr>
<td>Hypereosinophilic syndrome</td>
<td>Perianal fistulae</td>
<td></td>
</tr>
<tr>
<td>Juvenile cellulitis</td>
<td>Plasma cell pododermatitis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
An alternative method of classifying these diseases is to characterise them based on the underlying mechanism of action, as is shown in Table 2. This type of classification is interesting from an immunological perspective and it can be valuable in choosing an appropriate treatment regime, but it is still unhelpful from a diagnostic point of view.

Table 2.

<table>
<thead>
<tr>
<th>Antibody mediated</th>
<th>Lymphocyte mediated</th>
<th>Other cells/mixed/unknown</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyloidosis</td>
<td>Alopecia areata</td>
<td>Auricular chondritis</td>
</tr>
<tr>
<td>Bullous pemphigoid</td>
<td>Erythema multiforme</td>
<td>Dermatomyositis</td>
</tr>
<tr>
<td>Bullous SLE</td>
<td>Degenerative mucinotic mural</td>
<td>Granulomatous mural folliculitis</td>
</tr>
<tr>
<td>Cryoglobulinemia</td>
<td>folliculitis</td>
<td>Eosinophilic mucinotic mural</td>
</tr>
<tr>
<td>Drug eruption</td>
<td>Drug eruption</td>
<td>folliculitis</td>
</tr>
<tr>
<td>Epidermolysis bullosa (acquisita)</td>
<td>Follicular mucinosis</td>
<td>Histiocytosis</td>
</tr>
<tr>
<td>Linear IgA disease</td>
<td>Lupoid onychodystrophy</td>
<td>Hypereosinophilic syndrome</td>
</tr>
<tr>
<td>Lupus (systemic)</td>
<td>Lupus (cutaneous/DLE-vesicular/exfoliative)</td>
<td>Juvenile cellulitis</td>
</tr>
<tr>
<td>Mixed autoimmune</td>
<td>Lupus (systemic)</td>
<td>Lichenoid dermatoses</td>
</tr>
<tr>
<td>subepidermal blistering disease</td>
<td>Lymphocytosis/pseudolymphoma</td>
<td>Myospherulosis / Spherulocytosis</td>
</tr>
<tr>
<td>Mucous membrane pemphigoid</td>
<td>Perianal fistulae</td>
<td>Pyoderma gangrenosum</td>
</tr>
<tr>
<td>Pemphigus erythematosus</td>
<td>Plasma cell pododermatitis</td>
<td>Sarcoidosis</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
<td>Pseudopelade</td>
<td>Sterile eosinophilic pustulosis</td>
</tr>
<tr>
<td>Pemphigus vegetans</td>
<td>Sebaceous adenitis</td>
<td>Sterile granuloma/pyogranuloma syndrome</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td>Stevens Johnson syndrome</td>
<td>Sterile neutrophilic dermatitis</td>
</tr>
<tr>
<td>Paraneoplastic pemphigus</td>
<td>Toxic epidermal necrolysis</td>
<td>Sterile nodular panniculitis</td>
</tr>
<tr>
<td></td>
<td>Thymoma associated exfoliative dermatosis</td>
<td>Sterile pustular erythroderma</td>
</tr>
<tr>
<td></td>
<td>Uveodermatological syndrome</td>
<td>Subcorneal pustular dermatosis</td>
</tr>
<tr>
<td></td>
<td>Vitiligo</td>
<td>Vasculitis</td>
</tr>
</tbody>
</table>
Autoimmune skin diseases have also been characterised by the target antigen they recognise in
the body. For example:

- Desmocillon-1 - Pemphigus foliaceus
- Desmoglein-3 - Pemphigus vulgaris
- Nuclear antigens - Lupus
- Collagen XVII – Bullous pemphigoid
- Collagen XVII/Laminin 332 – Mucous membrane pemphigoid
- Collagen VII – Epidermolysis bullosa acquisita/bullous SLE

Again, this is interesting from a pathomechanistic perspective and in human medicine it
represents an important aspect of the diagnostic approach because autoreactivity to these
antigens can be detected using immunofluorescence testing. However, in the absence of widely
available immunological tests in veterinary medicine, it is currently not helpful for clinicians.

A method of classification that starts to become more clinically useful is to characterise these
diseases based on the structures in the skin they affect, as shown in Table 3. The value of this
approach is that it correlates with the changes seen on histopathological analysis. In other words,
if the skin is biopsied, identification of the affected structures can immediately help with
generation of a differential diagnosis. This would certainly help clinicians to perform clinic-
pathological correlation when they receive biopsy reports.

Table 3.

<table>
<thead>
<tr>
<th>Epidermis</th>
<th>Dermo-epidermal junction</th>
<th>Dermis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema multiforme</td>
<td>Bullous pemphigoid</td>
<td>Amyloidosis</td>
</tr>
<tr>
<td>Pemphigus erythematosus</td>
<td>Bullous SLE</td>
<td>Juvenile cellulitis</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
<td>Dermatomyositis</td>
<td>Sterile granuloma/pyogranuloma</td>
</tr>
<tr>
<td>Pemphigus vegetans</td>
<td>Epidermolysis bullosa (acquisita)</td>
<td>syndrome</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td>Lichenoid dermatoses</td>
<td>Histiocytosis</td>
</tr>
<tr>
<td>Paraneoplastic pemphigus</td>
<td>Linear IgA disease</td>
<td>Hypereosinophilic syndrome</td>
</tr>
<tr>
<td>Sterile eosinophilic pustulosis</td>
<td>Lupoid onychodystrophy</td>
<td>Lymphocytosis / pseudolymphoma</td>
</tr>
<tr>
<td>Sterile pupular erythroderma</td>
<td>Lupus (cutaneous/DLE)</td>
<td>Myospherulosis / Spherulocytosis</td>
</tr>
<tr>
<td>Stevens Johnson syndrome</td>
<td>Lupus (systemic)</td>
<td>Perianal fistulae</td>
</tr>
<tr>
<td>Subcorneal pustular dermatosis</td>
<td>Lupus (vesicular)</td>
<td>Plasma cell pododermatitis</td>
</tr>
<tr>
<td>Thymoma associated exfoliative dermatosis</td>
<td>Lupus (exfoliative)</td>
<td>Pyoderma gangrenosum</td>
</tr>
<tr>
<td>Toxic epidermal necrolysis</td>
<td>Mixed autoimmune subepidermal blistering disease</td>
<td>Sarcoidosis</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>Mucous membrane pemphigoid</td>
<td>Sterile neutrophilic dermatitis</td>
</tr>
<tr>
<td>Uveodermatological syndrome</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hair follicles

- Alopecia areata
- Degenerative mucinotic mural folliculitis
- Follicular mucinosis
- Granulomatous mural folliculitis
- Eosinophilic mucinotic mural folliculitis
- Pseudopelade

Subcutaneous fat

- Sterile nodular panniculitis

Sebaceous glands

- Sebaceous adenitis

Blood vessels

- Vasculitis
- Cryoglobulinaemia

Cartilage

- Auricular chondritis
Although this approach is very valuable from a pathological perspective, it still doesn’t help clinicians when they are first presented with a clinical case as the lesions that are seen and their distribution are very diverse. What is most beneficial is to classify these diseases based on the clinical lesions they cause. This approach, as shown in Table 4, allows clinicians to generate a differential diagnosis as soon as the animal is examined.

<table>
<thead>
<tr>
<th>Pustule/crusts</th>
<th>Ulceration</th>
<th>Alopecia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus erythematosus</td>
<td>Bullous pemphigoid</td>
<td>Alopecia areata</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
<td>Dermatomyositis</td>
<td>Degenerative mucinotic mural folliculitis</td>
</tr>
<tr>
<td>Pemphigus vegetans</td>
<td>Epidermolysis bullosa (acquisita)</td>
<td>Dermatomyositis</td>
</tr>
<tr>
<td>Sterile eosinophilic pustulosis</td>
<td>Lupus (cutaneous/DLE)</td>
<td>Eosinophilic mucinotic mural folliculitis</td>
</tr>
<tr>
<td>Subcorneal pustular dermatosis</td>
<td>Lupus (systemic)</td>
<td>Folliculitis</td>
</tr>
<tr>
<td>Sterile neutrophilic dermatitis</td>
<td>Lupus (vesicular)</td>
<td>Follicular mucinosis</td>
</tr>
<tr>
<td>Sterile nodular panniculitis</td>
<td>Mixed autoimmune subepidermal blistering disease</td>
<td>Granulomatous mural folliculitis</td>
</tr>
<tr>
<td>Sterile pustular erythroderma</td>
<td>Mucous membrane pemphigoid</td>
<td>Pseudopelade</td>
</tr>
<tr>
<td><strong>Swellings/lumps/nodules/discharging tracts</strong></td>
<td><strong>Depigmentation</strong></td>
<td><strong>Erythematous rash/purpura</strong></td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>Lupus (cutaneous/DLE)</td>
<td>Amyloidosis</td>
</tr>
<tr>
<td>Auricular chondritis</td>
<td>Perianal fistula</td>
<td>Erythema multiforme</td>
</tr>
<tr>
<td>Histiocytosis</td>
<td>Stevens Johnson syndrome</td>
<td>Hyperesinophilic syndrome</td>
</tr>
<tr>
<td>Juvenile cellulitis</td>
<td>Toxic epidermal necrolysis</td>
<td>Vasculitis</td>
</tr>
<tr>
<td>Lymphocytosis / pseudolymphoma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myospherosulosis / Spherulocytosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma cell pododermatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyoderma gangrenosum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoïdosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterile granuloma/pygranuloma syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Scaling</strong></td>
<td><strong>Erythematous rash/purpura</strong></td>
<td><strong>Depigmentation</strong></td>
</tr>
<tr>
<td>Lichenoid dermatoses</td>
<td>Amyloidosis</td>
<td>Lupus (cutaneous/DLE)</td>
</tr>
<tr>
<td>Lupus (exfoliative)</td>
<td>Erythema multiforme</td>
<td>Uveodermatological syndrome</td>
</tr>
<tr>
<td>Sebaceous adenitis</td>
<td>Hyperesinophilic syndrome</td>
<td>Vitiligo</td>
</tr>
<tr>
<td>Thymoma associated exfoliative dermatosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sloughing claws</strong></td>
<td><strong>Depigmentation</strong></td>
<td><strong>Erythematous rash/purpura</strong></td>
</tr>
<tr>
<td>Lupoid onychodystrophy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Out of this long list of diseases, there are some that are far more common than others. Whereas some would rarely be seen even by specialist dermatologists, others are common enough to be seen frequently in general practice. Two publications are particularly valuable for determining the prevalence of immune-mediated and autoimmune diseases. In 1987, Scott et al published a review of 9750 dogs and 2050 cats that had been presented to Cornell University with skin disease. Of these, 1.4% of the dogs and 1.3% of the cats had an immune-mediated skin disease. The five most common conditions diagnosed in dogs were pemphigus foliaceus, discoid lupus erythematosus, systemic lupus erythematosus, pemphigus vulgaris and vasculitis. In cats, pemphigus foliaceus, systemic lupus erythematosus, pemphigus vulgaris, erythema multiforme and pemphigus erythematosus were the most common. However, these cases were drawn from a referral population and they may not represent the true prevalences of immune-mediated conditions in the general population. In a more recent large scale survey of 27,035 diagnoses in kennel club registered breeds, the five most common immune-mediated skin diseases were symmetrical lupoid onychodystrophy, sebaceous adenitis, perianal fistulae, juvenile cellulitis and uveodermatological syndrome. Based on data such as this, and other unpublished observations, the conditions that general practitioners are most likely to see are the following:

- Pemphigus foliaceus
• Lupus (cutaneous/DLE)
• Juvenile cellulitis
• Perianal fistulae
• Vasculitis
• Lupoid onychodystrophy

The predominant clinical features of the above diseases and a summary of the treatment options, are shown in Table 5.

### Table 5.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Skin lesions</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pemphigus foliaceus</strong></td>
<td>Pustules and crusts on the nasal planum, nose, face, pinnae, trunk and footpads</td>
<td>Prednisolone, Azathioprine</td>
</tr>
<tr>
<td><strong>Cutaneous lupus/DLE</strong></td>
<td>Depigmentation, erosion, ulceration, crusts on the nasal planum, nose and other sites</td>
<td>Sun avoidance/topical sunscreens, Topical glucocorticoid, Topical tacrolimus/pimecrolimus, Doxycycline/niacinamide, Systemic immunosuppressive therapy</td>
</tr>
<tr>
<td><strong>Juvenile cellulitis</strong></td>
<td>Swelling and discharging tracts on the nose, lips, face, ears, distal limbs, Swollen submandibular lymph nodes</td>
<td>Prednisolone</td>
</tr>
<tr>
<td><strong>Perianal fistulae</strong></td>
<td>Ulcers, draining tracts on the perianal tissue</td>
<td>Ciclosporin, Tacrolimus</td>
</tr>
<tr>
<td><strong>Vasculitis</strong></td>
<td>Purpura, ulcers, infarcts, especially on extremities</td>
<td>Identify trigger, Manage wounds, Treat/prevent secondary infection, Pentoxyfylline, Immunosuppressive therapy, Surgery</td>
</tr>
<tr>
<td><strong>Lupoid onychodystrophy</strong></td>
<td>Sloughing claws</td>
<td>Fatty acids (Omega 3), Doxycycline/niacinamide, Pentoxyfylline, Ciclosporin, Immunosuppressive therapy, Surgical removal</td>
</tr>
</tbody>
</table>

Some other autoimmune or immune-mediated conditions that may be seen more commonly in specific breeds include the following:
• Lupus (vesicular) – collies and Shetland sheepdogs
• Lupus (exfoliative) – German shorthaired pointers
• Sebaceous adenitis - standard poodles, Samoyeds, Akitas, Vizlas, English springer spaniels
• Dermatomyositis – collies and Shetland sheepdogs
- Epidermolysis bullosa (acquisita) – young Great Danes
- Histiocytosis – multiple breeds, Bernese mountain dogs
- Plasma cell pododermatitis - cats
- Uveodermatological syndrome – Akitas, Australian shepherd dogs
AN UPDATE ON CUTANEOUS LUPUS ERYTHEMATOSUS

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Cutaneous lupus erythematosus (CLE) represents a group of benign autoimmune skin diseases of the dog, in which systemic manifestations are absent. The 1997 Gilliam-Sontheimer classification scheme for human cutaneous lupus diseases has recently been used as the basis for a reclassification of canine cutaneous lupus diseases. In this classification, canine cutaneous lupus diseases are divided into a subacute form, vesicular cutaneous lupus erythematosus (VCLE) and a number of chronic cutaneous lupus erythematosus (CCLE) diseases: these are exfoliative CLE (ECLE), mucocutaneous lupus erythematosus (MCLE), and discoid LE (DLE), which is further divided into a generalised form (GDLE) and the facially predominant “classic” DLE (FDLE). Of these, FDLE is by far the most common, usually being reported as the second most common cutaneous autoimmune disease in the dog, after pemphigus foliaceus. However, in tropical and subtropical climates, FDLE may be the most common form.

The pathogenesis of CLE is poorly understood. A complete understanding of the interactions between genetics, immune-system and environmental triggers remains elusive. However, it is thought that UV light plays a role in the genesis of both canine FDLE and human CLE, and UV light is known to be an exacerbating factor in the former. UV light induces cytokine release and apoptosis of keratinocytes. It is suggested in human beings that accumulation of apoptotic keratinocytes in the skin leads to uptake of apoptotic debris by antigen presenting cells, resulting in auto-antibodies against epidermal antigens. A similar process may occur in the skin of dogs with some forms of CLE.

Facial Discoid Lupus Erythematosus (FDLE)
Clinical signs of FDLE involve depigmentation of the nasal planum and loss of the surface “cobblestone” appearance, which may progress to scaling, crusting and ulceration of the planum and adjacent skin. Spontaneous bleeding may be a feature. Diagnosis of FDLE is made on the basis of history, clinical appearance and supportive histopathological findings of a lichenoid infiltrate with lymphocytes, macrophages, plasma cells and fewer neutrophils, with hydropic degeneration and/or apoptosis of the basal cell layer and pigmentary incontinence. The significance of pigmentary incontinence in biopsies from nasal epithelium is questionable. The differential diagnosis list includes actinic dermatitis, nasal depigmentation, vitiligo, pemphigus foliaceus, pemphigus erythematosus, trauma, dermatomyositis, lymphoma, drug reaction, uveodermatoologic syndrome, contact dermatitis and systemic lupus erythematosus. Mucocutaneous pyoderma has long been held to be a significant differential but close analysis of the literature suggests that this may not in fact be the case. In the majority of cases, the clinical appearance of FDLE is quite typical and it is a matter of some debate as to whether biopsy is necessary. Collies, Shetland sheepdogs, German shepherd dogs and Siberian huskies have been reported as being at greater risk of developing the disease. In our practice, border collies are also over-represented. Diagnosis of other forms of CLE relies on histopathological analysis, because the clinical appearance of these cases is less typical.

Antibiotics of the tetracycline family combined with niacinamide have been suggested as being useful in the treatment of canine immune-mediated skin diseases, including FDLE. As well as
anti-microbial activity, tetracyclines have been shown to have anti-oxidant, anti-apoptotic and anti-inflammatory effects. Anti-inflammatory activity includes inhibition of pro-inflammatory cytokines such as tumor necrosis factor α, interleukin 1β and interleukin 6, and inhibition of pro-inflammatory proteins including inducible nitric acid synthase (iNOS), cyclo-oxygenase 2 and matrix metalloproteinases. Niacinamide has been shown to have anti-inflammatory and photo-protective effects. Synergism of tetracyclines and niacinamide is widely suggested but unproven. Doxycycline may be preferred to tetracycline as it can be administered once or twice daily, whilst tetracycline requires three times a day dosing. Examination of records at our practice shows that doxycycline/niacinamide treatment has a positive response rate of approximately 80% in FDLE. Response to therapy may be slow, with at least six to twelve weeks being required before maximal effects are seen.

Glucocorticoids, usually oral prednisolone, are also effective for FDLE. Because of their side effect potential, their use is usually reserved for refractory cases and situations where economic considerations preclude the use of other medications. Glucocorticoids may also be useful for short term therapy at the initiation of other forms of treatment, to carry patients over any lag phase. This can be helpful for initial treatment of severe cases where spontaneous bleeding is a feature.

The calcineurin inhibitor cyclosporin (with or without the addition of ketoconazole as a cyclosporin sparing agent) is an effective treatment for refractory FDLE. Calcineurin is a key enzyme in T-cell activation. In addition, cyclosporin decreases the number of Langerhan’s cells in the dermis and inhibits some antigen presenting functions. Cyclosporin is well-tolerated in most patients. Gastrointestinal side effects are common on initiation of therapy, with up to 25% of patients affected, but most resolve after a short time. In the author’s experience, it is rare to have to discontinue treatment with cyclosporin due to unacceptable side effects. Time to response with cyclosporin appears to be similar to its use in other diseases, with maximal effects being noted in four to eight weeks.

Recently, the Janus Kinase inhibitor oclacitinib has been proposed as a treatment for this and other autoimmune diseases, through its modulatory effects on T-lymphocytes. Whilst there are currently no large-scale peer-reviewed clinical papers to support its use for FDLE, our practice has recently started to use oclacitinib as first line treatment for this disease. Initial responses appear to be favourable and we look forward to presenting data on this treatment during the presentation. Oclacitinib is well-tolerated by most patients.

Topical therapies, including topical glucocorticoids, tacrolimus and pimecrolimus have also been advocated for the treatment of FDLE. There is anecdotal evidence to support their use, but in our practice they are rarely useful. This may be due to our high UV environment, where the benefits of therapy may be overwhelmed by ongoing UV damage. Dogs also lick off topical treatment as soon as it is applied, regardless of owner efforts to prevent this. Similarly, the use of topical sunscreens on affected areas does not appear to confer much benefit. However, these are benign treatments and so their use, particularly in mild cases, may be worthwhile.

Because FDLE is a UV exacerbated, if not initiated, disease, sun avoidance is key to successful treatment. In sunny climates this is rarely enough on its own to induce and maintain remission.
However, avoidance of UV exposure forms a key element in any successful treatment regime. Sunscreen use is often ineffective for the reasons enumerated above. Restricting affected dogs to shaded areas or providing physical UV protection for the nasal planum is more likely to be helpful. Home-made “nose-caps” or commercially available products may be helpful.

Other Forms of Canine CLE

*Generalised Discoid Lupus Erythematosus* is a rare disease characterised by generalised or multifocal discoid plaques demonstrating cycles of ulceration followed by healing and scarring. Most cases have lesions affecting the trunk but mucocutaneous junctions may also be involved. There is variable pruritus and pain at lesion sites. Any breed can be affected but German shepherd dogs appear to be under-represented. Diagnosis is based on histopathology with (usually) well developed reactions as described for FDLE. Cyclosporin would appear to be the treatment of choice for this disease.

*Vesicular Cutaneous Lupus Erythematosus* is classified as a subacute CLE and onset of lesions is faster than in other diseases in this group. Most cases in the literature are middle age to older collies and shelties, but the majority of cases seen in our practice have been border collies. Cases present with erythema and flaccid vesicles on the glabrous skin of the abdomen and associated areas, which progress to focal to confluent serpiginous areas of ulceration. Mucocutaneous junctions may be affected but not usually as severely as the abdomen. Diagnosis based is based on histopathology with an interface dermatitis with basal cell vacuolation and apoptosis. The pattern here is usually more florid than FDLE and can be severe enough to cause intrabasal clefting. However, “cell-poor” areas can lead to confusion with dermatomyositis. Other differentials for this disease may include sub-epidermal autoimmune blistering diseases. Treatment with prednisolone (+/- azathioprine) has been described but cyclosporin appears to be the treatment of choice.

*Exfoliative Cutaneous Lupus Erythematosus* (ECLE) is a severe genetic disease of young German short haired pointers. It is inherited in an autosomal recessive manner. The disease has also been reported in a group of viszlas. Clinical signs consist of generalised scaling and alopecia with or without pruritus. Histopathology reveals multifocal lichenoid dermatitis with basal cell apoptosis, extending into the follicular epithelium in the majority of cases. Sebaceous glands are also often affected. Treatment is often unrewarding and response to therapy is poor. Hydroxychloroquine may slow the progression of disease in some cases. Because of the young age of onset and the known genetic nature of the disease, this is not a frequently encountered disease.

*Mucocutaneous Lupus Erythematosus* (MCLE) is a disease characterised by ulcerative lesions at mucocutaneous junctions, with the anus and peri-genital skin most commonly affected. Lesions on the periocular skin, nasal planum and lips are also reported. 50% of reported cases have been in German shepherd dogs. Diagnosis is based on histopathology, which is similar to FDLE and the changes may be mild or patchy. Treatment with prednisolone is reportedly effective with fast remission of signs. Most cases at our practice have been treated with doxycycline and niacinamide to good effect.
Selected References

LOKIVETMAB – DOES IT DO MORE THAN TREAT ATOPIC ITCH?

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Lokivetmab: what is it?
Lokivetmab is an immunoglobulin G class monoclonal (therapeutic) antibody that targets interleukin 31 (IL-31). Mice are injected with canine IL-31 to induce production of anti-IL-31 antibodies. Antigen specific plasma cells are harvested from the mouse and hybridised with a neoplastic immune cell for immortality. The immortalised hybrid cells are then cloned for production. The monoclonal antibody is caninised so has the structure of the canine IgG with complementarity determining regions (CDRs) made up of murine origin (this is the bit that binds the IL-31). Immunogenicity is reduced by the caninisation BUT immunogenicity is NOT just due to the murine component. Thus, removal of murine proteins does NOT remove all immunogenicity but does improve tolerance.¹,²,³

Lokivetmab pharmacokinetics
The distribution and elimination of monoclonal antibodies are complex with numerous patient factors and disease factors (target concentration) making duration of action unpredictable. A major pathway for elimination is pinocytosis uptake and degradation in endothelial cells (especially skin, gut and muscles) for IgG monoclonals not undergoing receptor mediated elimination. Once the monoclonal enters the cell and enters the endosome, acidification in the endosome increases affinity for IgG to bind to neonatal FcR receptor. This prevents degradation and returns the IgG to the surface and re-enters circulation. In people there is documented genetic variation in FcR receptor preservation and this markedly affects the monoclonal half-life. On average, two-thirds of IgG is preserved and the half-life extended to 18 to 21 days. Based on what is known on therapeutic monoclonal antibody pharmacokinetics in people, IL-31 levels (target mediated drug disposition), FcR receptor levels, catabolic state of the patient, inflammatory state of the skin at site of injection (proteases), obesity, cutaneous mucinosis, patient activity level at time of injection and location of the injection can all potentially affect tissue distribution and degradation. Development of neutralising antibodies by the patient can cause lack of target binding (neutralising antibodies directed to the murine CDRs result in sudden loss of efficacy) or increased rate of degradation and reduced duration of action (neutralising antibodies may prevent binding to the neonatal FcR and thus are not preserved from degradation and are not recycled therefore reducing the half-life).¹

Lokivetmab action
Lokivetmab binds circulating (and tissue) IL-31, prevents IL-31 binding to its’ IL-31 receptor and thus prevents the induction of IL-31 mediated itch.⁴ IL-31 is a major trigger of pruritus in canine atopic dermatitis.⁵ IL-31 is a relatively new player in dermatology. The question is what else does IL 31 do? What other potential actions does lokivetmab have via its’ specific ability to block IL-31?

Could lokivetmab prevent the development of canine atopic dermatitis (CAD)? The p53 family member, ΔNp63 isoform, has a key role in driving keratinocyte activation in human AD. Overexpression of ΔNp63 in transgenic mouse epidermis results in a severe skin phenotype that
shares many of the key clinical, histological and molecular features associated with human AD. This includes deviation of Th-2 inflammatory cascade. One of the target genes for p63 is IL-31. Elevated levels of ΔNp63 isoform occurs in atopic human skin resulting in increased IL-31 and IL-33. This is pivotal to atopic progression. IL-31 and IL-33 are downstream regulators of increased ΔNp63 expression. IL-31 signals via a heterodimeric receptor IL-31 R and the Oncostatin M receptor (OSMR). All 3 are upregulated via upregulation of ΔNp63 and this precedes the onset of itch. IL-31 upregulation results in a greater than 2 fold increase in expression of more than 570 genes, many of which are associated with epidermal differentiation and formation of the epidermal barrier. IL-31 is central to the current outside-inside-outside theory of pathogenesis in AD.6,7 Less is known about the role of IL-31 in the progression of canine AD. In a canine breeding colony with known prevalence of AD, it would be an intriguing study to use lokivetmab proactively during early life and see whether this alters the prevalence.

**Could lokivetmab prevent repeat flares of CAD?**

IL-31 is upregulated early and is the most upregulated cytokine at 6 and 24 hours after epicutaneous challenge with *D. farinae* in sensitised dogs.8 Proactive use of lokivetmab can inhibit pruritic responses to allergen challenge in experimental models of CAD and in spontaneous CAD was able to prevent flares of CAD, for up to 12 months, in approximately a quarter of dogs when used as a monotherapy.9 Lokivetmab used proactively for 6 months in 81 dogs that were lokivetmab responders, maintained control based on visual analogue scale in 76% (68/81) of dogs and based on CADESI 03 in 59% (48/81) of patients.10 Atopic inflammation involves numerous inflammatory pathways. A narrow, targeted therapy like lokivetmab would not be expected to rescue an atopic flare but, once inflammation is controlled, lokivetmab may be effective in preventing atopic flares in a subset of atopic dogs. Higher level of pruritus control compared to lesion control reflects its’ targeted action. Variation in efficacy probably reflects the wide genotypic/phenotypic variation in CAD.

**Could lokivetmab improve barrier function in dogs with CAD?**

Increased IL-31 expression is associated with increased severity of AD and increased inhibition of keratinocyte differentiation leading to down regulation of epidermal barrier function. IL-31 affects keratinocyte differentiation in multiple ways and the IL-1 cytokine network is a major downstream effector of IL-31 signalling in deregulating the physical skin barrier. However, low doses of IL-31 promote the antimicrobial barrier (production of antimicrobial peptides) so complete inhibition of IL-31 signalling may be undesirable (may increase microbial colonisation). IL-31 inhibition in CAD could potentially improve the physical skin barrier but reduce the antimicrobial barrier function.7 Proactive use of lokivetmab in canine atopic dermatitis did not significantly reduce the rate of skin and ear infections in spite of good pruritus control.10 This is probably a reflection of lokivetmab’s targeted action and lack of control of atopic inflammation.

**Could lokivetmab reduce the severity of allergic conjunctivitis in dogs with CAD?**

A murine study looked at the role of IL-31 in allergic conjunctivitis. Cedar pollen sensitised mice were exposed conjunctivally to cedar pollen alone or cedar pollen plus IL-31. The IL-31 augmented challenges showed significantly elevated mastocytic and eosinophilic inflammation.11
Could lokivetmab improve the hair coat of dogs with CAD?
Over-expression of IL-31 has been shown to cause reversible alopecia (in people). 12

Could lokivetmab be effective for allergic contact dermatitis?
In an experimental model for allergy contact dermatitis, IL-31 deficient mice developed lesions on challenge as per normal mice but the pruritus was prevented.13

Could lokivetmab be effective in managing itch in dogs with cutaneous infections, mast cell neoplasia, cutaneous T-cell lymphoma?
Any disease process where IL-31 is a major mediator of itch should be improved by lokivetmab. Paraneoplastic pruritus with cutaneous T-cell lymphoma and mastocytosis have been shown to involve IL-31.14,15 Cutaneous infections can trigger liberation of IL-31 from dermal mast cells.16

Lokivetmab and cancer?
IL-31R is expressed in a number of mouse and human cancer cell lines (including breast, colon, mast cell, follicular lymphoma and hematological malignancies). IL-31 expression has an anti-tumour effect on solid tumours associated with anti-angiogenic effects, while promoting growth of haematological neoplasia. Hence the potential effects, desirable or undesirable, may depend on the tumour type. At this time, it is unknown whether IL-31 plays similar roles in canine neoplasia. Until more is known about the role of IL-31 in dogs with cancer, lokivetmab therapy in the presence of neoplasia should be evaluated carefully.17

Lokivetmab for inflammatory bowel disorder?
In people, IL-31 has been shown to be over-expressed in the intestinal mucosa in Crohn’s disease and ulcerative colitis. IL-31 is pro-inflammatory and anti-proliferative and degrades the integrity of the intestinal mucosa.18 Could this be the case in dogs as well?

Conclusion
As we understand more about the role of IL-31 in atopic and non-atopic diseases in the dog, there may be further indications (and contraindications) for use of lokivetmab.

References


ADVANCED IMMUNOSUPPRESSIVE THERAPY
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Introduction
In the veterinary dermatology specialty, we are often confronted with a variety of diseases that require the use of immunosuppressive therapy. These disorders include autoimmune skin diseases (pemphigus complex, lupus erythematosus complex, mucous membrane pemphigoid, bullous pemphigoid and uveodermatitis syndrome), histiocytic and sterile pyogranulomatous inflammatory diseases, idiopathic vasculitis and some cases of erythema multiforme and drug reactions. These disorders can be extremely challenging to treat and manage. Glucocorticoids are often used in many of these diseases because of their immediate impact on the immune system by reducing inflammation in a non-specific fashion by inhibiting gene transcription, intracellular signaling pathways, downregulating the expression of adhesion molecules and slowing cell proliferation. In some disorders, glucocorticoids work well enough and at a low enough dosing that they can be used as a monotherapy with little to no side effects. In many cases, the use of long-term glucocorticoids creates significant side effects and the need for additional immunosuppressive options or multimodal immunosuppressive therapy is required. It is beyond the scope of this discussion to cover all possible alternatives to glucocorticoids and topics will be confined to the more commonly used drugs in clinical practice.

Azathioprine
Azathioprine (Imuran®, Glaxo Wellcome and generics) is a derivative of 6-mercaptopurine (6-MP). It has a wider therapeutic index than the other thiopurine drugs. It is most practitioners’ first choice immunosuppressive to use in pemphigus, other autoimmune diseases, sterile pyogranulomatous disorders, histocytic diseases and vasculitis cases. Azathioprine is an antimetabolite that interferes with the synthesis of nucleic acids and is cytotoxic to T cells. It has its greatest effect on T cell dependent antibody synthesis. Because of its mechanism of action, it has a slow onset of action and usually takes 4 to 8 weeks to see clinical effects.

The thiopurines are pro-drugs and have to be metabolized in order to exert their cytotoxic action. Azathioprine is non-enzymatically reduced to 6-mercaptopurine (MP) and an imidazole group. Both 6-MP and 6-thioguanine (TG) undergo extensive metabolism before exerting cytotoxicity following incorporation into DNA as thioguanine nucleotides (TGNs) or in the case of 6-MP inhibition of de novo purine synthesis (DNPS). The TGNs are incorporated instead of the bases into the DNA and cause DNA-protein cross-links, single strand breaks, interstrand cross-links and sister chromatid exchanges. This incorporation of TGNs into DNA has been shown to be recognized by the mismatch repair (MMR) system. Defects in the MMR pathway have been shown to be associated with resistance to 6-TG. The main enzymes competing for the initial metabolism of 6-MP and 6-TG are thiopurine methyltransferase (TPMT), HGPRT, aldehyde oxidase, and xanthine oxidase (XO). Due to genetic polymorphisms, TPMT activity varies in different individuals and species. It is the main enzyme responsible for the metabolism of azathioprine into inactive 6-methylmercaptopurine. This inter-individual variation in activity greatly influences cytotoxicity of the thiopurine drugs. For example, azathioprine is contraindicated in cats due to its more profound myelosuppression and potential for fatal reactions. This may relate to the low levels of TPMT activity in cats. There are variations in the
activity levels of this enzyme between dog breeds as well. Giant schnauzers have much lower
TPMT activity (and therefore are more susceptible to azathioprine related toxicity) and Alaskan
malamutes had much higher TPMT activity (and thus perhaps needing higher dosages of
azathioprine). The metabolism of 6-MP to TGMP can lead to the production of S-methyl-
thioinosine 5-monophosphate (MeTIMP), which is believed to exert a significant contribution to
the cytotoxic action of 6-MP. Azathioprine is dosed at 1.5 – 2.5mg/kg q 24h - 48h. Some
specialists prefer calculation of dosing in mg/m² (50 mg/m² PO q12-48h). It is available as a
50mg scored tablet (Glaxo Wellcome, Faro Pharmaceuticals and generics).

Adverse reactions primarily include myelosuppression (lymphopenia, anemia, thrombocytopenia
and leukopenia), diarrhea and increased susceptibility to opportunistic infections when used
long-term (pyoderma, demodicosis, dermatophytosis). Diarrhea usually responds to dosage
reduction, although it can be severe (hemorrhagic) and require drug discontinuation. Less
common complications include vomiting, hepatotoxicity and pancreatitis. The hepatotoxicity
usually responds to drug reduction or withdrawal. Dosage adjustments can be made based on the
results of lab monitoring and clinical improvement. Starting at the lower end of the dosage
range is generally recommended. Increasing the dosage after subsequent rechecks and lab monitoring
can be performed as the case progresses. Complete blood counts with platelet counts are
recommended every 2 to 3 weeks for the first 3 months of therapy. Platelets are produced in high
volume daily; therefore, thrombocytopenia can be the first indication of bone marrow
suppression. However, in some situations lymphocyte numbers can be initially impacted, as this
is often the first cell line to show reduction even before platelet counts reductions. Initially,
periodic (every 4 weeks) monitoring of chemistry profiles is also recommended to watch for
hepatotoxicity. Monitoring can be reduced to every 6 months once cases are in remission.

Chlorambucil
Chlorambucil (Leukeran®, Glaxo Wellcome) is an alkylating agent that functions by affecting
the cross linking of DNA. It is considered less toxic and slower acting than other alkylating
agents. Chlorambucil can be used in the dog as a glucocorticoid-sparing drug, as an alternative to
azathioprine or in combination with glucocorticoids and azathioprine in more refractory cases. In
patients that don’t tolerate other therapies, chlorambucil can be tried as sole therapy. Indications
are similar to azathioprine. It is also a drug of choice in feline pemphigus. Chlorambucil is an
orally administered alkylating agent that is cytotoxic due to cross-linking of DNA. It disturbs
DNA synthesis and cellular proliferation. It is not cell cycle specific and acts most readily on
rapidly dividing cells but exerts some influence on cells not actively synthesizing DNA. It is
slow acting and less toxic than other alkylating agents. It is dosed at 0.1 -0.2-mg/kg or 15mg/m²
q 24h to 48h. It is available in a 2 mg non-scored coated tablet (Leukeran®, Glaxo Wellcome),
making dosing in small dogs and cats possible.

Myelosuppression is a concern and similar monitoring as listed with azathioprine should be
performed. Other side effects include vomiting, diarrhea and anorexia. Anorexia, vomiting and
diarrhea will often resolve with alternate day dosing. Anecdotal reports of alopecia and delayed
hair regrowth after clipping that may be seen more commonly in poodles and Kerry blue terriers.
**Lomustine (CCNU)**

Lomustine (CCNU) is an alkylating agent belonging to the nitrosourea group. It is initially hydroxylated intracellularly by hepatic microsomal (P450) enzymes to form an alkylating molecule. It inhibits DNA and RNA synthesis via carbamylation of DNA polymerase, alkylation of DNA and alteration of RNA, proteins and enzymes and is cell cycle non-specific. CCNU is absorbed completely from GI tract and can appear in plasma within 3 minutes of administration. It is metabolized in the liver by hydroxylation (produces at least 2 active metabolites). Peak metabolite concentrations reach 3 to 4 hours in human beings. It is highly lipid soluble causing wide distribution to tissue and ability to penetrate the blood-brain barrier. CCNU is excreted through the kidneys with small amounts through bile and enterohepatic circulation.

This drug is most commonly used in cases of epitheliotropic lymphoma and occasionally in mast cell tumors and histiocytic sarcoma. The dosage ranges from as low as 30 - 60 mg/m² every 3 to 6 weeks in dogs. Starting with lower dosages is often not only safer but cases will respond to this dose and exhibit less side effects. Hemograms and chemistry screens should be performed every 2 to 3 weeks prior to given subsequent dosing. The main side effects include myelosuppression, renal toxicity, gastrointestinal toxicity and hepatotoxicity.

**Tetracycline and Niacinamide**

The combination of tetracycline and niacinamide has been used with variable success in dogs and human beings with pemphigus. Tetracycline and niacinamide is more beneficial as adjunctive therapy in the pemphigus complex but can be more efficacious for localized cases such as pemphigus erythematosus. The best reported successes are in cases of discoid lupus erythematosus. This combination has also been used in idiopathic sterile pyogranulomatous dermatoses, histiocytic dermatoses, vasculopathies, lupoid onychodystrophy and arteritis of the nasal philtrum. Doxycycline has also been used in place of tetracycline. In the cat, doxycycline alone is helpful in some cases of plasmacytic pododermatitis and plasmacytic stomatitis.

Although the exact mechanism of action regarding anti-inflammatory or immunomodulatory effects is not known, both drugs have benefits in these areas. Tetracycline has anti-inflammatory properties affecting complement activation, antibody production, chemotaxis, prostaglandin synthesis, lipases and collagenases and suppressing lymphocyte blastogenesis. Niacinamide blocks antigen and IgE-induced histamine release in-vivo and in-vitro and inhibits mast-cell degranulation and phosphodiesterase protease release. The dosage recommendations are 500mg of each drug q 8h for dogs weighing more than 10kg and 250mg q 8h for dogs weighing less than 10kg. Tetracycline can be replaced with doxycycline at 5mg/kg a 12h. Clinical response may take 1 –2 months. If clinical response is seen the frequency can be reduced to twice or even once daily. If doxycycline is used in place of tetracycline, the dose is 10mg/kg q 24h.

Adverse reactions include vomiting, diarrhea, anorexia and increased liver enzymes. When gastrointestinal complications occur, discontinuing the niacinamide may reduce or eliminate these problems. In rare cases the tetracycline may still produce beneficial results. Owners and pharmacists show be cautioned about not using niacin in place of niacinamide, as it is not well tolerated and can create significant gastrointestinal upsets and possible hepatotoxicity at higher dosages. Tetracycline should not be used in young dogs prior to complete adult teeth have developed as it has been associated with dental enamel hypoplasia. Doxycycline can create
esophageal erosions and ulceration and needs to used carefully especially in the feline, where liquid formulations followed by water ingestion is recommended.

**Cyclosporine and Tacrolimus**

Cyclosporine (Atopica®, Novartis) and tacrolimus (Prograf® oral formulation and Protopic® 0.1% and 0.03% topical preparations, Fujisawa USA, Inc) are immunosuppressant agents that have been used extensively in human medicine, primarily to prevent organ transplant rejection. These drugs have received most of their veterinary attention for treatment of atopic dermatitis and perianal fistulas, but many other uses have been found for these drugs and as an alternative for other immunosuppressive therapies. Conditions reported or suggested include various forms of cutaneous lupus erythematosus, eosinophilic granuloma complex in the cat, feline pseudopelade, feline atopic dermatitis, pemphigus erythematousus, pemphigus foliaceus, sebaceous adenitis, sterile nodular panniculitis, chronic pedal furunculosis, dirty face syndrome of Persian cats, erythema multiforme, primary seborrhea and/or epidermal, dysplasia, end-stage proliferative otitis externa, German Shepherd dog deep pyoderma, metatarsal fistula, ulcerative dermatosis of the philtrum of St. Bernard and Newfoundland dogs, feline plasma cell stomatitis and pododermatitis. The initial studies of cyclosporine (CsA) in the treatment of pemphigus and other cutaneous autoimmune diseases in dogs have not been impressive and only limited responses have been seen. In these early studies, older non-microencapsulated formulations of cyclosporine were utilized. Much better responses utilizing the microencapsulated formulation have been reported. Depending upon the disease and severity of the disease, dosages range from 5-10mg/kg q 24h. It is also common to use CsA in conjunction with oral glucocorticoids. However, it may be used as a sole agent or as a glucocorticoid sparing agent as well. CsA has been used with other immunosuppressive agents including azathioprine for cases of refractory pemphigus foliaceus with some initial success.

Cyclosporine is also available as a topical 0.2% ophthalmic ointment (Optimmune, Schering-Plough). Some localized forms of discoid lupus erythematosus (DLE) and pemphigus erythematousus (PE) may respond to this being used as an adjunctive agent. However, topical administration of 0.1% tacrolimus has been much more efficacious especially in cases of DLE and PE. Both of these drugs work similarly, however tacrolimus is much more potent and the oral formulations appear toxic in the dog. Due to the much greater potency and potential toxicity of tacrolimus and lack of adequate dosing regimes, systemic administration is not recommended in canine clinical cases.

Both drugs become activated by binding to specific intracellular receptors, called immunophilins. Cyclosporine binds to cyclophilin and tacrolimus binds to FK506-binding proteins. Both drugs inhibit calcium-dependent pathways, particularly affecting the enzymatic actions of calcineurin, a calmodulin-dependent protein phosphatase. Calcineurin is a calcium and calmodulin dependent protein phosphatase that is responsible for transmission of signal information from the cell membrane to the nucleus. Calcineurin transmits information by dephosphorylating nuclear factor of activated T cells (NF-AT). NF-AT is a transcription factor that penetrates the nucleus and induces cell activation. One of the essential processes in cellular activation is the transcription of genes controlling the synthesis of IL-2. Inhibition of IL-2 transcription and T-cell responsiveness to IL-2 results in impaired T-helper and T-cytotoxic lymphocytes. Inhibition or impaired production of other cytokines, such as interferon-α (IFN-α),
IL-3, IL-4, IL-5, and TNF-α, has an impact on mononuclear cell function, mast cell and eosinophil production and survival, cytoplasmic granule release, cytokine secretion, prostaglandin production, neutrophil adherence, NK cell activity, and growth and differentiation of B cells. In addition, CsA decreases the number of Langerhans cells in the epidermis, inhibits the lymphocyte-activating function of Langerhans cells, and diminishes cytokine secretion by keratinocytes. Because of its lipophilic properties, CsA is widely distributed in most tissues, but has low passage across the blood-brain barrier. CsA is metabolized by the cytochrome P450 enzyme system, specifically CYP3A4, in the liver and intestines. Drugs that inhibit CYP3A4 or compete with P-glycoprotein may alter CsA metabolism substantially.

Although numerous medications have been documented to affect CsA metabolism in human beings, few clinically relevant drug interactions have been reported in veterinary medicine. Ketoconazole represents one of these important and useful drug interactions. Ketoconazole suppresses the cytochrome P450 enzymes, thereby decreasing CsA clearance and increasing CsA serum concentrations. Concurrent administration of CsA and ketoconazole can produce CsA serum levels double of what can be seen with just CsA alone. Use of the two together (CsA at 5mg/kg q 24h and ketoconazole 2.5mg/kg q 24h) has been noted to result in similar serum levels of what CsA at 10 mg/kg q 24h produces alone. This can also result in substantial cost savings to the client. Atopica® (Novartis) is the only FDA approved veterinary microemulsion formulation that improves absorption of the drug and is available in capsules of 10mg, 25mg, 50 mg and 100mg. The absorption of the drug can be variable; Atopica® and Neoral® (the human form similar to Atopica) are more readily absorbed than its predecessor Sandimmune® (Novartis). Atopica® is most consistently absorbed when given without food on an empty stomach. When given with food in some dogs, the incidence of vomiting and other gastrointestinal problems may be reduced. Several microemulsion generic products have become available and they usually work as well as brand name drugs.

While human beings experience a high incidence of nephrotoxicity and hepatic toxicity, this is not commonly observed in dogs. Dogs may experience gingival hyperplasia and papillomatosis, vomiting, diarrhea, bacteriuria, bacterial skin infection, anorexia, hirsutism, involuntary shaking, nephropathy, bone marrow suppression, and lymphoplasmacytoid dermatosis. Side effects seen with cyclosporine most commonly include anorexia, vomiting or diarrhea. Vomiting can respond to dosage reduction, dividing dosing up throughout the day or giving the drug with food, although as noted this can reduce effective serum levels. A variety of products to control vomiting have been recommended, the most effective is maropitant citrate (Cerenia, Pfizer) 2 mg/kg/d. Other side effects that occur with some frequency but are not serious include gingival hyperplasia and hirsutism. These both usually respond to dosage reduction or discontinuation of the drug. In a limited number of cases viral cutaneous lesions can occur, some of these have been associated with papillomavirus antigens. Interestingly, the incidence of lymphoma in human beings treated with CsA maybe associated with the higher incidence of Epstein Barr virus infections. In dogs, there are a few anecdotal cases of lymphoma and other round cell tumors reported (plasmacytomas and histiocytomas). Rare cases of systemic atypical deep mycotic infections with Prototheca, Aspergillossis and Phaeohyphomycosis species have also been reported. However, these have been seen most often in dogs that were on other potent high-dose immunosuppressive drugs such as concurrent high-dose glucocorticoids.
Long term cyclosporine pharmacovigilance studies showed adverse events (AE) were rare (71.81 AEs/million capsules sold). Gastrointestinal reactions were most common, but were mild and rarely required intervention. Other AEs were rare (<1/10 per cent in clinical trials; <10/million capsules sold). Hirsutism, gingival hyperplasia and hyperplastic dermatitis were rarely significant and resolved on dose reduction. CsA decreases staphylococcal and Malassezia infections in atopic dermatitis (AD), and at the recommended dose is not a risk factor for other infections, neoplasia, renal failure or hypertension. Concomitant treatment with most drugs is safe. Because CsA can also inhibit the p-glycoprotein pump, caution should be used in treating animals with cyclosporine and avermectins concurrently. Side effects in cats are currently considered rare. Concerns have arisen about increased susceptibility to viral and other latent infections. Fatal acute systemic toxoplasmosis has recently been diagnosed in a limited number of cases.

The topical tacrolimus preparations appear very safe. However, there are concerns associated with increase risk for neoplasia in human beings. A black box warning from the FDA regarding the potential risk for developing neoplasia under treatment with calcineurin inhibitors has made patients and physicians uncertain about its safety, regardless of the fact that current scientific data does not support increased concern for risk of malignancy. Because of this concern, it is recommended that clients apply it with gloves. Monitoring serum levels of both cyclosporine and tacrolimus have been evaluated. In the authors experience, the serum levels of cyclosporine generally do not correlate with clinical responses. However, there may be some limited value to monitor these levels when clinical responses are not seen or if toxicity is suspected. If levels are still in the low therapeutic range dosages could be increased in an attempt to obtain a response. The author commonly performs pre-cyclosporine lab work and monitors cases on an every 6-month basis with complete blood counts, chemistry profiles and urinalyses. There may be a tendency for urinary tract infections. However, many dogs that have developed cystitis have been on glucocorticoid therapy previously.

**Mycophenolate mofetil**

Mycophenolate mofetil (CellCept®, Roche Pharmaceuticals) is a drug that inhibits *de novo* purine (guanine) synthesis. In human beings it has been used in a variety of autoimmune skin diseases, myasthenia gravis, autoimmune hepatitis and immune cytopenias, inflammatory bowel disease and prevention of allograft rejection. Canine studies in pemphigus foliaceus show success rates of approximately 50% with some dogs weaned completely off prednisone, while others have relapsed when the glucocorticoid dosages were lowered. Mycophenolate mofetil is prodrug of the antiproliferative agent mycophenolic acid (MPA). It specifically and reversibly inhibits inosine monophosphate dehydrogenase thereby blocking synthesis of guanine. The drug blocks production of guanosine 5 phosphate, interferes with *de novo* purine synthesis and blocks the precursor production for DNA and RNA synthesis. It is cytotoxic to cells that rely on *de-novo* purine synthesis (proliferating T and B lymphocytes) rather than purine salvage pathway (myeloid cells). It also inhibits antibody formation and generation of cytotoxic T-cells. MPA also can induce T-lymphocyte apoptosis. MPA suppresses dendritic cell maturation and can induce human monocyte-macrophage cell line differentiation, decreasing the expression of interleukin (IL)-1 and enhancing expression of the IL-1 receptor antagonist. It is available in 250mg and 500mg tablets. Dosages range from 22-39 mg/kg q 24h divided q 8h.
The main side effects include bone marrow suppression, nausea, vomiting, diarrhea, and increase incidence of infections. Other side effects were minimal but the most common include pyoderma and *Malassezia* infections, diarrhea and leukocytosis. Expense was a limiting factor in the past but generics are now available making it a very cost effective option.

**Pentoxifylline**

Pentoxifylline (Trental, Hoechst-Roussel and generics) is a methylxanthine derivative that produces a variety of physiologic changes at the cellular level. Pentoxifylline has also been used for a variety of inflammatory diseases such as necrobiosis lipoidica, granuloma annulare and brown recluse spider bites. The drug also affects wound healing and connective tissue disorders through increased production of collagenase and decreased production of collagen, fibronectin, and glycosaminoglycans. Because of this, it has been used in human beings to treat scleroderma. Pentoxifylline has been used in veterinary medicine for the treatment of canine familial dermatomyositis, allergic contact dermatitis and canine atopic dogs. Anecdotal and case reports indicate beneficial effects in treating pinnal thrombovascular necrosis, ear-margin dermatosis, vasculitis, rabies vaccine–related alopecia, erythema multiforme, drug reactions, idiopathic mucinosis of the Chinese Shar Pei, lupoid onychodystrophy, vesicular cutaneous lupus erythematosus, epitheliomatous lupus erythematosus, epitheliomatous lymphoma, acral lick dermatitis, Greyhound vasculopathy and metatarsal fistulae of German Shepherds.

Immunomodulatory and rheologic effects include increased leukocyte deformability and chemotaxis, decreased platelet aggregation, decreased leukocyte responsiveness to interleukin-1 (IL-1) and tumor necrosis factor (TNF)-α, decreased production of TNF-α from macrophages, decreased production of IL-1, IL-4, and IL-12, inhibition of T- and B-lymphocyte activation, and decreased natural killer cell activity. It also has been shown to inhibit T-cell adherence to keratinocytes. Dosing is variable ranging from 10-30 mg/kg q8h to q 12h, depending on the size of the dog and the disease being treated. A pharmacokinetic study in dogs showed that 30mg/kg oral dosing should be effective, as therapeutic human concentrations (1,000 ng/ml) were reached and persisted for 510 minutes (+/- 85min). Some question whether the use of generic forms of the drug are as good as the brand name, but no comparative studies exist regarding differences in efficacy or metabolism. Once there is a favorable response, which may take 1 to 3 months, the dose can be tapered to either q12h or q24h depending upon what starting dosage was utilized. Some cases require the q8h dosing to remain effective.

In general, no serious effects have been reported in dogs. Vomiting, diarrhea, dizziness and headaches are the major concerns and are dose related in people. Two dogs on pentoxifylline reportedly developed erythema multiforme. This is particularly interesting, as the drug has been used to treat erythema multiforme successfully. Monitoring while on long-term therapy is variable. Most cases should be checked annually and routine lab screening.

**Leflunomide**

Leflunomide is a synthetic isoxazole derivative that is metabolized to its active metabolite mononitrilamide or teriflunomide. Teriflunomide primarily functions as a selective pyrimidine synthesis inhibitor via the reversible inhibition of dihydroorotate dehydrogenase, a mitochondrial enzyme required for de novo pyrimidine biosynthesis. It may also affect tyrosine kinase activity, which alters cytokine and growth factor receptors.
associated with tyrosine kinase activity. Leflunomide targets B lymphocytes and T lymphocytes, which lack a pyrimidine salvage pathway. Leflunomide has been used in people for its immunosuppressive and antiproliferative effects in the treatment of rheumatoid arthritis, Crohn’s disease, systemic lupus erythematosus and the management of transplant recipients. It has been used in combination therapy for a number of immune mediated/inflammatory disease in dogs, including immune-mediated thrombocytopenia, immune-mediated hemolytic anemia, systemic histocytosis, nonsuppurative encephalitis/meningomyelitis, immune-mediated polymyositis, immune-mediated polyarthritis, pemphigus foliaceus, reactive histocytosis and immune-mediated polyarthritis. Leflunomide is dosed in the dog at 2-4mg/kg q 24hr. Side effects in dogs include lethargy, gastrointestinal upset, mild bone marrow suppression (leukopenia and thrombocytopenia) and rarely hepatoxicity and TEN. Similar monitoring for bone marrow suppression and hepatotoxicity should be performed as with other bone marrow suppressive and hepatoxic drugs.

**Hydroxychloroquine**
Hydroxychloroquine (Plaquenil) is classified as an anti-malarial drug. It is useful in treating several forms of malaria as well as lupus erythematosus and rheumatoid arthritis in human beings. In people, it has slow onset of action (6–8 weeks) and functions by downregulating the immune response. Specifically, it increases the pH in cellular compartments and thereby interferes with the formation of peptide-MHC protein complexes which are needed to stimulate CD4+ T cells. Serious side effects of hydroxychloroquine in people are uncommon. An irreversible retinopathy is rare, and risk factors include long-term therapy (usually >5 years) at high doses, advanced age and renal insufficiency. Other adverse effects are reversible corneal lipid deposits, irritability, hyperactivity, seizures, vomiting/diarrhea, reversible hematological abnormalities and cutaneous eruptions. Cardiotoxicity is also rare and more likely to occur in patients with SLE. It has been used in two previous reports for treating lupus in dogs. The first cases involved use for treating exfoliative cutaneous lupus erythematosus in three German shorthaired pointers at a dose of 5-10mg/kg q 24hr; there was no improvement or adverse reactions noted. In the second report, it was used in a form of generalize discoid lupus erythematosus at dose of 5mg/kg q 24hr and where the dog had an excellent response to this treatment regime.

**Oclacitinib**
Janus kinase (JAK) inhibitors are a class of drugs which interfere with the JAK-STAT signaling pathway and initially were used for the treatment of various cancers, notably myeloproliferative neoplasms in people. They also inhibit various cytokines IL2, IL4, IL5, IL15 and IL21 and block T-helper cell differentiations and have been used in rheumatoid arthritis and allergic diseases. There are reports in human and canine autoimmune diseases including alopecia areata, dermatomyositis and SLE, with oclacitinib reported to be affected in a case of canine subepidermal bullous dermatitis and ischemic dermatitis. There have been numerous anecdotal reports of good responses in cases of pemphigus foliaceus and vasculitis. Dermatologists report that oclacitinib seems to be most effective in combination therapy with glucocorticoids, usually at dosages of 0.6mg/kg q 12h. The addition of oclacitinib has allowed better control and often reduction in glucocorticoid dosing in some cases. While using and maintaining higher dosing of oclacitinib, other JAK 1 and JAK 2 sensitive cytokines include erythropoietin (EPO) and granulocyte and macrophage colony

172
stimulating factor (GM-CSF) can be affected. Other similar side effects as reported in the use for patients with atopic dermatitis can be seen. More frequent monitoring for potential side effects should be performed when treating autoimmune disorders at higher dosing. The author commonly screens complete blood counts, serum chemistry profiles and urinalysis every 3 months in cases maintained on 0.6mg/kg q12h.

Selected References
**OCLACITINIB – CAN IT DO MORE THAN TREAT ATOPIC DERMATITIS?**

Greg Burton and Mark Walczak  
Animal Skin & Ear Specialists  
Melbourne Veterinary Specialist Centre, Melbourne, Australia

**Oclacitinib—what is it?**  
Oclacitinib is a novel janus kinase inhibitor (JAKi) developed for the control of cytokine signalling associated with pruritus and inflammation induction in canine atopic dermatitis. Oclacitinib is specific for inhibiting cytokines that signal via the JAK/STAT pathway. Oclacitinib inhibited JAK family members by 50% at concentrations (IC50's) ranging from 10 to 99 nM but did not inhibit a panel of 38 non-JAK kinases (IC50’s >1000 nM). Oclacitinib was most potent at inhibiting JAK1 (IC50=10 nM). Oclacitinib also inhibited the function of JAK1-dependent cytokines involved in allergy and inflammation (IL-2, IL-4, IL-6, and IL-13), as well as, pruritus (IL-31) at IC50’s ranging from 36 to 249 nM. Oclacitinib had minimal effects on cytokines that did not activate the JAK1 enzyme in cells (erythropoietin, granulocyte/macrophage colony-stimulating factor, IL-12, IL-23 (IC50’s >1000 nM)). Oclacitinib did not inhibit T-cell clonal expansion or T-cell cytokine synthesis at 1000nM (did at 10000nM). The absolute bioavailability of oclacitinib maleate was found to be 89%. The major clearance mechanism for oclacitinib is metabolism. Cmax was 324 ng/ml = 960nM. Tmax is 0.9 hours; T ½ is 4.13 hours.

**Could oclacitinib be useful for immune mediated disease?**  
Upregulation of the JAK/STAT pathway or dysfunction of intracellular (suppression) regulation of those pathways has been recognised in a number of immune mediated diseases in people. Interferon γ (IFNγ), IL15 and IL2 are upregulated in alopecia areata and cutaneous lupus. IFNγ acts via JAK1 and 2, and IL15 and IL2 via JAK1 and 3; therefore, JAK1 inhibition is potentially useful. IFNs and IL-15 have also been implicated in immune mediated induction of apoptosis and IFNs in ischaemic cytopathy. IL-15 serum levels have been shown to correlate with severity and mortality in people with Steven Johnson Syndrome. JAK1 activation is seen in vitiligo and is associated with IFNγ (CD 8 T-cell) induced melanocyte destruction.

In people, pemphigus is associated with accumulation of autoreactive B cells in the skin and the skin acts a tertiary lymphoid organ. Close interaction between IL-21 and IL-17A producing CD4+ T-cells results in local production of anti-desmoglein autoantibodies. IL-21 secretion is strongly associated with JAK1 activation and tofacitinib (JAK1 and JAK3 inhibitor) has been suggested for human pemphigus vulgaris (PV) and pemphigus foliaceous (PF). While the specific target may differ in canine PF, if the process of autoantibody production is similar to people, then JAK1 inhibition may be helpful in canine PF.

JAK inhibitors have been used effectively in people in the following diseases: alopecia areata, atopic dermatitis, graft versus host disease, lupus erythematosus, psoriasis, dermatomyositis, vitiligo and Steven Johnson syndrome. Oclacitinib has been used effectively in dogs with atopic dermatitis and ischaemic dermatopathy.

The Melbourne Veterinary Specialty Center has evaluated the use of oclacitinib for immune mediated disease including facial discoid lupus erythematosus (DLE), pemphigus foliaceous...
(PF), ischaemic dermatopathy, canine vesicular cutaneous lupus erythematosus (VCLE), symmetrical lupoid onychodystrophy (SLOD), Steven Johnson Syndrome (SJS) and vitiligo. A summary of clinical cases and recommendations for these diseases are as follows.

Table 1. Discoid lupus erythematosus

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Breed</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Dose of Oclacitinib (mg/kg)</th>
<th>Response</th>
<th>Duration of Oclacitinib therapy</th>
<th>Concurrent treatment</th>
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<td>FS</td>
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<td>Maintenance (q24h)</td>
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<td>Partial</td>
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P=1% Pimecrolimus

Outcomes and recommendations for DLE

Complete resolution was defined as 100% resolution of erosions and crusts, and partial or complete re-pigmentation. Partial resolution was defined as greater than 80% resolution of erosions and crusts with partial re-pigmentation. Of the 12 cases with greater than 4 month follow up, 5/12 (41.6%) showed complete resolution (2/5 had concurrent topical pimecrolimus twice daily). 7/12 (58.4%) showed greater than 80% resolution (2/7 had concurrent topical pimecrolimus twice daily). Adequate control was achieved in all cases with once daily oclacitinib for maintenance. Concurrent, effective sun-avoidance was key for complete resolution.
### Table 2. Pemphigus foliaceous

<table>
<thead>
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<th>Case no.</th>
<th>Breed</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Dose of Oclacitinib (mg/kg)</th>
<th>Response</th>
<th>Duration of Oclacitinib therapy</th>
<th>Concurrent treatment</th>
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<td>MN</td>
<td>0.41</td>
<td>Complete</td>
<td>13 months</td>
<td>0.17mg/kg P EOD</td>
</tr>
<tr>
<td>4</td>
<td>Kelpie x Rottweiler</td>
<td>10.1</td>
<td>FS</td>
<td>0.51 (6/7 days)</td>
<td>Complete</td>
<td>16 months</td>
<td>Topical M q24h</td>
</tr>
<tr>
<td>5*</td>
<td>German Sheppard x Chow Chow</td>
<td>6</td>
<td>FS</td>
<td>0.54</td>
<td>Complete</td>
<td>14 months</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Min Dachshund</td>
<td>7.4</td>
<td>FS</td>
<td>0.42</td>
<td>Partial</td>
<td>4 months</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Maltese X</td>
<td>5</td>
<td>FS</td>
<td>0.51</td>
<td>None</td>
<td>14 days</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Cocker Sp</td>
<td>7.1</td>
<td>FS</td>
<td>0.54</td>
<td>None</td>
<td>14 days</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Jack Russel X</td>
<td>13.1</td>
<td>MN</td>
<td>0.6</td>
<td>None</td>
<td>3 days</td>
<td></td>
</tr>
</tbody>
</table>

* Maintained on oclacitinib q12h. P = prednisolone, M = 1% mometasone furoate

**Outcomes and recommendations for PF**

Complete resolution was defined as 100% resolution of erosions and crusts. Partial resolution was defined as greater than 80% resolution of erosion and crusts. Therapy was withdrawn if there was no clinical improvement after 14 days of twice daily therapy (or in case 9 after 3 days due to rapid progression). Three of nine cases (33.3%) showed no response. Six of nine cases (66.6%) showed good response with 5/6 (83.3%) of the responders showing complete resolution. Oclacitinib can be recommended as a first-line treatment for PF, while awaiting histological confirmation of the diagnosis. All responders showed rapid improvement. Two of 6 responding cases required twice daily oclacitinib maintenance for control.

### Table 3. Ischaemic Dermatopathy

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Breed</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Dose of Oclacitinib (mg/kg)</th>
<th>Response</th>
<th>Duration of Oclacitinib therapy</th>
<th>Concurrent treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1*</td>
<td>Dalmatian</td>
<td>6.7</td>
<td>MN</td>
<td>0.6</td>
<td>None</td>
<td>2 weeks</td>
<td>Topical P q24h</td>
</tr>
<tr>
<td>2</td>
<td>Sharpei</td>
<td>5.2</td>
<td>FS</td>
<td>0.37</td>
<td>Complete</td>
<td>38 months</td>
<td>13.7mg/kg Pt q12h</td>
</tr>
<tr>
<td>3</td>
<td>Borzoi</td>
<td>4.3</td>
<td>FE</td>
<td>0.47</td>
<td>Complete</td>
<td>22 months</td>
<td>17mg/kg C</td>
</tr>
<tr>
<td>4</td>
<td>Collie x Kelpie</td>
<td>0.7</td>
<td>FS</td>
<td>0.6</td>
<td>Partial</td>
<td>14 months</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Rhodesian Ridgeback</td>
<td>2.6</td>
<td>FS</td>
<td>0.6</td>
<td>Complete</td>
<td>19 months</td>
<td>Topical P q24h</td>
</tr>
<tr>
<td>6</td>
<td>Husky</td>
<td>5.6</td>
<td>MN</td>
<td>0.57</td>
<td>Complete</td>
<td>6 weeks</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Dalmation</td>
<td>5.10</td>
<td>MN</td>
<td>0.55</td>
<td>Partial</td>
<td>10 months</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Kelpie</td>
<td>0.8</td>
<td>FS</td>
<td>0.61</td>
<td>Complete</td>
<td>4 months</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Great Dane</td>
<td>6</td>
<td>MN</td>
<td>0.45</td>
<td>Partial</td>
<td>22 months</td>
<td>-</td>
</tr>
</tbody>
</table>

P = pimecrolimus, Pt = pentoxifyline, C = colchicine

**Outcomes and recommendations for ischaemic dermatopathy**
Complete resolution was defined as 100% resolution of active lesions (erosions and crusts). The presence of cicatricial alopecia was not considered as an incomplete outcome. Cicatricial alopecia is a permanent sequela and was often present at time of referral, prior to start of oclacitinib therapy. Therapy was withdrawn if no clinical improvement seen after 14 days of twice daily therapy. One of 9 cases (11.1%) showed no clinical response (this was a case of pinnal vasculitis). Five of 8 responders (62.5%) showed complete resolution. Two of the 8 complete responders were adverse drug reactions and required no long term maintenance, while 6/8 could be maintained on once daily dosing. Case 8 had histological evidence of vasculitis and SJS. Three of 8 cases (37.5%) were partial responders on once daily therapy but responded completely on twice daily dosing. Oclacitinib appears effective for the management of ischaemic dermatopathies.

**Table 4. Canine Vesicular Lupus Erythematosus**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Breed</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Dose of Oclacitinib (mg/kg)</th>
<th>Response</th>
<th>Duration of Oclacitinib therapy</th>
<th>Concurrent treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial (q12h)</td>
<td>Maintenance (q24h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Border Collie</td>
<td>8</td>
<td>FS</td>
<td>0.57</td>
<td>0.57</td>
<td>Complete</td>
<td>11 months</td>
</tr>
<tr>
<td>2</td>
<td>Border Collie</td>
<td>7.4</td>
<td>FS</td>
<td>0.54</td>
<td>0.54</td>
<td>Partial</td>
<td>2.5 months</td>
</tr>
<tr>
<td>3</td>
<td>Border Collie</td>
<td>3.10</td>
<td>F</td>
<td>0.4</td>
<td>0.25</td>
<td>Complete</td>
<td>10 months</td>
</tr>
<tr>
<td>4</td>
<td>Border Collie</td>
<td>12.6</td>
<td>FS</td>
<td>0.57</td>
<td>0.57</td>
<td>Complete</td>
<td>13 months</td>
</tr>
</tbody>
</table>

**Outcomes and recommendations for VCLE**

Complete resolution was defined as 100% resolution of active lesions (macular or serpiginous, erythema, erosions and crusts). Partial response was defined as greater than 80% resolution of active lesions. All cases showed good clinical outcome with three-quarters showing compete resolution. All cases could be maintained on once daily oclacitinib. Oclacitinib appears to be the treatment of choice for this disease.

**Table 5. Symmetrical lupoid onychodystrophy**

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Breed</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Dose of Oclacitinib (mg/kg)</th>
<th>Response</th>
<th>Duration of Oclacitinib therapy</th>
<th>Concurrent treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial (q12h)</td>
<td>Maintenance (q24h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Labrador Retriever</td>
<td>8.8</td>
<td>FS</td>
<td>0.5</td>
<td>0.5</td>
<td>Partial</td>
<td>5 months</td>
</tr>
<tr>
<td>2*</td>
<td>Staffordshire Bull Terrier</td>
<td>6.1</td>
<td>MN</td>
<td>0.56</td>
<td>-</td>
<td>None</td>
<td>14 days</td>
</tr>
<tr>
<td>3</td>
<td>Miniature Dachshund</td>
<td>6.1</td>
<td>FS</td>
<td>0.57</td>
<td>0.57</td>
<td>Complete</td>
<td>12 weeks</td>
</tr>
<tr>
<td>4*</td>
<td>Labrador Retriever</td>
<td>3.2</td>
<td>FS</td>
<td>0.51</td>
<td>0.51</td>
<td>None</td>
<td>3 months</td>
</tr>
</tbody>
</table>

*Therapy discontinued due to lack of improvement of symptoms. R robenacoxib, N niacinamide.

**Outcomes and recommendations for symmetrical lupoid onychodystrophy**

Of the 4 SLOD cases treated with oclacitinib, only 1 case showed adequate response. Oclacitinib cannot be recommended for the treatment of SLOD.

**Outcomes and recommendations for SJS and vitiligo?**

A single case of drug-induced SJS responded rapidly and spectacularly to oclacitinib 0.61mg/kg bid and medication was withdrawn after 4 months with complete resolution. A single case of
vitiligo with rapid progression of pigment loss has shown a cessation of progression after 14 days on oclacitinib 0.5mg/kg bid at the time of writing. Longer term follow up will be included in the accompanying presentation.

**Oclacitinib and cancer?**

**Solid tumors**
- Oclacitinib would be expected to inhibit IL-15 via its’ inhibition of JAK 1
- IL-15 in lymphocytes activates JAK1/STAT3 and JAK3/STAT
- IL-15 is central to the development, survival and activation of natural killer (NK), T-, and B-cells. IL-15 increases Natural Killer (NK) cell production of perforins and granulozymes A and B responsible for induction of cell death. Upregulation of NK cells by IL-15 important in immune responses to solid tumors. IL-15 therapy is being explored for solid tumors. IL-15 does NOT increase regulatory T cells (IL-2 does). Increased T regs are undesirable in therapy as it leads to immune suppression/tolerance to the tumor. Blocking the action of IL-15 would be undesirable in solid tumors. Solid tumors may also develop JAK1 loss of function mutations as part of immune avoidance.  
- IL-31 signaling is also blocked by JAK1 inhibition. IL-31 has antiangiogenic effect on solid tumours. 
- Based on these findings, oclacitinib is best avoided in patients with solid malignancies

**Lymphoid tumors**
- IL-15 is a growth and viability factor for malignant T cells including epitheliotropic lymphoma
- IL-15 has a strong epidermotropism effect due to aberrant expression of IL-15 in keratinocytes in epitheliotropic lymphoma via JAK I and JAK 3 activation. IL-15 is a potent chemottractor of malignant T cells to the skin and stimulates malignant cutaneous T-cell lymphoma (CTCL) cells to produce anti-apoptotic bcl2 protein.
- IL-15 promotes survival of large granular cell leukemia
- JAK1 inhibition is useful for management of IL-31 mediated paraneoplastic itch.
- Oclacitinib may be useful palliation for epitheliotropic lymphoma due to anti IL-15 and IL-31 effects
Table 6. Epitheliotropic lymphoma (authors experience)

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Breed</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Dose of Oclacitinib (mg/kg)</th>
<th>Response</th>
<th>Duration of Oclacitinib therapy before relapse</th>
<th>Concurrent treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Beagle</td>
<td>11</td>
<td>FS</td>
<td>0.52</td>
<td>0.52</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>2*</td>
<td>Great Dane</td>
<td>7</td>
<td>FS</td>
<td>0.6</td>
<td>0.6*</td>
<td>Partial</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Maltese X</td>
<td>13</td>
<td>FS</td>
<td>0.45</td>
<td>0.45*</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Boxer X</td>
<td>12</td>
<td>FS</td>
<td>0.5</td>
<td>0.5</td>
<td>Partial</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>WHW</td>
<td>12.7</td>
<td>MN</td>
<td>0.6</td>
<td>0.6*</td>
<td>Good</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Gordon Setter</td>
<td>12.1</td>
<td>MN</td>
<td>0.46</td>
<td>0.46*</td>
<td>Partial</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Jack Russell</td>
<td>10.5</td>
<td>MN</td>
<td>0.62</td>
<td>0.62*</td>
<td>Poor</td>
<td>DNR</td>
</tr>
<tr>
<td>8</td>
<td>Boxer</td>
<td>8.2</td>
<td>MN</td>
<td>0.41</td>
<td>0.41*</td>
<td>Good</td>
<td>10 weeks</td>
</tr>
</tbody>
</table>

Outcomes and recommendations for epitheliotropic lymphoma.

Good outcome was defined as greater than 80% resolution of clinical lesions and pruritus (if present). Partial response was defined as a 50 to 80% resolution in clinical lesions and pruritus. A poor response was defined as a 0 to 49% resolution of clinical lesions. One of 8 dogs failed to show any response and the disease progressed on twice daily oclacitinib for 8 weeks. Four of 8 cases (50%) showed good responses for 6 to 18 months before relapse. Relapse generally involved development of plaque or nodular disease, as well as, recurrence of exfoliative erythroderma and erosive disease. Three of 8 cases (37.5%) showed partial responses with progression of disease occurring after 4 to 5 months of treatment. Six of the 8 cases required twice daily maintenance with worsening of symptoms when reduced to once daily dosing.

References

ADVERSE DRUG REACTIONS IN DOGS AND CATS

Stephen D. White
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University of California, Davis, CA, USA

Introduction
Adverse drug reaction may refer to either a known adverse effect of a medication, due to its understood mechanism of action, molecular structure or other intrinsic determinants; or an adverse reaction may be idiopathic, without a clear link to the medication’s known properties. As an example, injectable amikacin is known to have the adverse effect of renal damage; a dog receiving those injections that developed a severe erythrodema with pruritus that resolved upon cessation of the drug would illustrate the idiopathic type of adverse drug reaction. Ultimately, all adverse drug reactions could probably fit into the known reason category; we just do not know enough about all drugs’ properties and how they might react in individual animals’ genetic make-up. This lecture will focus on adverse drug reactions affecting the skin or medications used for skin diseases that subsequently affect the animal.

Guidelines for Evaluating a Potential Adverse Drug Reaction
1. The more recent a medication’s administration is, the more likely it is the cause. For example, if a cat has been receiving cyclosporine for 2 years for its atopic dermatitis, then last week it received an antibiotic, and now it has a papular-macular eruption, the closer temporal association between the antibiotic and the eruption would make the antibiotic more likely than the cyclosporine. Of course, other causes of such a reaction would also have to be considered, such as a flea allergy.

2. Extrapolating from reports in humans, the more common groups of medications that cause adverse drug reactions affecting the skin in small animals probably are the potentiated sulfas, the semi-synthetic penicillins, non-steroidal anti-inflammatory drugs (NSAIDs), anti-cancer medications, and possibly biologics.

3. It is often easy to focus on a drug for causing an unknown/unanticipated adverse reaction, but in many cases this is a diagnosis of exclusion. The veterinarian should conduct a thorough investigation of other causes before settling on a medication as the cause.

4. Recall that many owners do not think of over-the-counter products as ‘drugs’. Thus vitamins, nutritional supplements, shampoos or other topical products may all go unreported unless the veterinarian specifically asks about these substances in the history.

5. Never try to test the theory of an adverse drug reaction by re-administrating the drug! There is a very good likelihood that the second administration will cause a worse reaction. This also means avoiding all similar medications. A Naranjo scoring method may be used to assess the possibility of a medication having caused an adverse reaction.

6. Treatment is identifying and stopping the causative drug, accompanied in most cases with immune-suppression, usually with corticosteroids, occasionally with other immunosuppressive agents. If a topical product is suspected, rinse the animal off with lukewarm or cool water, and if
possible use a shampoo whose contents are different from the offending product. If only a small area is affected, or if the animal will not tolerate bathing, a damp washcloth may be used. Also, any hair with visible product should be clipped off.

**Reported Adverse Reactions**

Some reported examples of potentially known mechanisms causing adverse drug reactions:  
1. *Phenobarbital* administration adversely affecting the liver and subsequently associated with superficial necrolytic dermatitis.  
2. *Cyclosporine* suppressing the immune system in cats prior to exposure to *Toxoplasma gondii* with subsequent disease caused by the protozoa.  
3. Drugs with *sulfhydryl* groups causing pemphigus foliaceus (as has been theorized in people).

Examples of reported adverse reactions without a clear link to the known properties of the drugs:  
1. *Vaccine reactions*. These have been seen in both dogs and cats. In dogs, rabies vaccinations have caused ischemic dermatitis, and in cats they have been linked to proliferative and necrotizing otitis externa.  
2. *Methimazole* can cause a facial dermatitis that bears some resemblance to erythema multiforme (see below) and has also been reported to cause a pyogranulomatous dermatitis.  
3. *Orbifloxacin* has been suspected of causing a cutaneous drug reaction in one series of dogs treated with the drug.  
4. *Itraconazole* and *ivermectin* were associated with cutaneous vasculitis in a retrospective review.  
5. *Sterile neutrophilic dermatitis* (Sweet’s-like syndrome) is a non-infectious, presumed immune mediated condition typified by erythema, fever, malaise, neutrophilia, lameness and neutrophilic effusions into the joints. This is often a rapidly progressive disease, and may be caused by certain medications (NSAIDs have been hypothesized), as well as arising spontaneously. Diagnosis is based on skin biopsy, the lack of bacteria on culture of intact skin lesions or joint aspirates.  
6. *Sterile Pustular Erythroderma of Miniature Schnauzers* A rare, severe, often fatal disease, seemingly limited to miniature Schnauzers, often preceded by bathing. In one abstract, a contact reaction to one of the components in an aloe-based shampoo was implicated. This condition presents with severe depression and malaise, often with fever. Skin lesions are (often dramatic) erythema, pustules or epidermal collarettes, and/or wheals. These dogs are very sick – treatment, when successful, consists of high dose corticosteroids.  
7. *Well’s like syndrome* is an eosinophilic, generalized dermatitis to cellulitis. It has been associated with either gastrointestinal signs and/or drugs (metronidazole?) in some, but not all cases. Peripheral eosinophilia is rare. Skin lesions are papules, macules, and erythema. Pruritus is variable. Histopathology shows an eosinophilic dermatitis, often with eosinophilic ‘flame figures’ (collagen surrounded by eosinophils or their granules). Treatment consists of prednisolone/prednisone at 1-2 mg/kg, then tapered. Treatment duration is variable, but should be continued for at least one month.

*Erythema multiforme* (EM) is an acute eruption of the skin and mucous membranes. While EM has been reported to have an association with drug eruptions, recent work points to TEN (see below) and ‘cross-over syndromes’ between the two diseases as more likely to be due to drug involvement. It is characterized in human beings clinically by annular (“target”) lesions. While these have been observed in dogs, more common signs are mucocutaneous vesicles, ulcers,
maculae, and/or urticarial plaques may also be seen. In widespread lesions, the ventrum and periocular areas are often involved. EM may be self-limiting, although by the time the animals arrive at the specialist’s office, this is not often the case. EM histologically shows apoptotic (programmed-cell death mechanism-activated) keratinocytes, with satellitosis (lymphocytes surrounding the keratinocytes, presumably triggering the programmed cell death mechanism). The author has seen a few cases that seemed to have an ischemic component or occurred concurrently with an ischemic dermatitis. While theoretically pentoxifylline should be helpful, the author has seen 2 dogs which had EM induced by pentoxifylline! Intravenous human immunoglobulin has been reported as successful in two dogs when infused on 2 consecutive days (1 g/kg per day). This has also been reported to be successful in a cat. However, it is a relatively expensive treatment. While immune-suppression is the usual recommendation, the author has seen one cat in which the EM was probably related to concurrent sepsis.

Toxic epidermal necrolysis (TEN) is a rare vesiculobullous and ulcerative disease of the skin and mucosa of dogs and cats. It is characterized by an acute onset of pyrexia, anorexia, lethargy, and/or depression, and a multifocal or generalized vesiculobullous eruption. The outer layer of the skin adjacent to lesions may be easily rubbed away (positive Nikolsky sign). Pain is moderate to severe. Histopathology includes intracellular edema (hydropic degeneration) of the basal epidermal cells, full thickness epidermal necrosis, and minimal dermal inflammation. Dermoepidermal separation may be present. It is important to realize that TEN often seems to be associated with drug eruption. Therapy involves treating the underlying disease, symptomatic and supportive therapy, and corticosteroids.

Selected References


THE APPROACH TO AN UNUSUAL SKIN LESION OR DISEASE

Peter B. Hill
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This lecture and notes will describe the author’s approach when evaluating an unusual looking skin lesion or disease. As this presentation is very visual in nature, these notes are intentionally brief and spaces are provided for additional notes to be made about the various examples used.

Clinicians are readily able to recognise commonly seen skin lesions such as erythema, pustules, alopecia, crusts, scaling and ulcers. But what if the nature of these lesions or their distribution appears unusual? What if the animal before you looks like nothing you have seen before?

When presented with such a case, the author adopts a step-by-step approach to try and determine logically what the problem might be. This approach is as follows:

1. Can I match what I see with a known entity or something that I have seen before? This approach relies on pattern recognition. The extent to which this approach can be used is dependent on the experience of the clinician. An experienced general practitioner would be able to use this approach more than a veterinary student or new graduate. However, even with experience, this approach can fail if the practitioner is presented with an uncommon or rare disease. However, even with such rare cases, a specialist dermatologist may be able to utilize this approach because they are more likely to have seen it before, or at least have read about it.

   Examples of cases used to illustrate this approach can be recorded here.

2. From the history and clinical signs, can I generate a list of differentials comprising known entities? This is essentially the problem-oriented approach. In this situation, it isn’t possible to make a definitive diagnosis just on the history and clinical signs of the patient, but it is possible to come up with a list of possible causes.

   Examples of cases used to illustrate this approach can be recorded here.
3. Can I use probability to inform my differential diagnosis? This is an extension of the problem-oriented approach that allows prioritization of the differential diagnosis list. This approach again depends on the experience and expertise of the clinician because the knowledge required to generate the probabilities can be obscure or specialized.
   *Examples of cases used to illustrate this approach can be recorded here.*

4. Can I figure it out by looking at the lesions themselves? This approach requires a detailed knowledge of the pathogenesis of skin lesions. What can actually make skin become red and what does it mean if red skin is not itchy? What mechanisms can make skin become black or white and what pathology is likely to result in this? What can make lesions appear circular or in straight lines?
   *Examples of cases used to illustrate this approach can be recorded here.*

5. If that still doesn't help, is there a human condition that looks like this grossly.
   *Examples of cases used to illustrate this approach can be recorded here.*

6. If the lesion morphology doesn't help me, I turn to histopathology. Histopathology is a very useful tool for the diagnosis of unusual skin lesions or diseases. However, there are a number of pitfalls when submitting samples for histopathology that clinicians need to be aware of.
   - Gross vs Microscopic pathology
     It is important to remember that the clinician is able to observe the entire range of pathologies that are present on the skin. The pathologist is usually presented with a very small sample of these changes. Thus, it is crucial that the lesions that are selected for biopsy are representative of the underlying disease process.
   - Limited repertoire of responses
The skin is only able to respond to a diverse range of etiological causes in a limited number of ways. Hence, histopathology can be inconclusive if the etiological cause is not apparent on the biopsies.

- **Mimicry**
  Some skin diseases can mimic other ones both grossly and histopathologically.

- **Focal pathology**
  The diagnostic changes in a biopsy can be very focal in nature. Much of the pathology seen may be non-specific and the diagnostically relevant part may only represent a small component of the samples and therefore the biopsy report.

To overcome some of these problems, it is essential that clinico-pathological correlation is performed. This means that the diagnosis is based on a combination of information derived from the history and clinical signs, rather than just the biopsy report.

*Examples of cases used to illustrate this approach and pitfalls can be recorded here.*
GENERAL DIAGNOSTIC APPROACH TO PRURITIC SKIN DISEASE IN CATS

Danny W. Scott
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Many dermatoses can rarely or commonly cause pruritus in cats. However, over one-third of the cats presented to dermatology services have allergic skin disease. Hence, this discussion will focus on the differential diagnosis of the “allergic-like” cat.

There are four classic cutaneous reaction patterns in the allergic cat: (1) initially lesionless symmetrical pruritus (especially face, ears, and/or neck), (2) self-induced symmetrical hair loss (especially ventral abdomen, medial thighs, legs, dorsal lumbosacral region), (3) symmetrical papulocrustous (“miliary”) dermatitis (especially dorsum, neck), and (4) eosinophilic granuloma complex (indolent ulcer [especially lip], eosinophilic plaque [especially ventral abdomen, medial thigh], eosinophilic granuloma [especially lip, chin, caudal thigh]). Over one-third of allergic cats manifest two or more of these reaction patterns at the time of presentation.

However, these cutaneous reaction patterns are also seen in nonallergic dermatoses. Initially lesionless symmetrical pruritus may also be seen with dacryocystitis, otodectic mange (“ear mites”), trombiculidiasis (“chiggers”), and early notoedric and sarcotic mange (“scabies”). Self-induced symmetrical hair loss may be seen with certain ectoparasitisms (fleas, cheyletiellosis, otodectic mange, *Demodex gatoi* infestation), hyperthyroidism, and behavioral over-grooming (very rare). Symmetrical papulocrustous dermatitis may also be seen with ectoparasitisms (cheyletiellosis, otodectic mange, *Lynxacarus radovskyi* infestation, pediculosis [lice], trombiculidiasis), staphylococcal folliculitis and dermatophytosis. Eosinophilic granuloma complex lesions may be seen with certain staphylococcal infections, ectoparasitisms, contact dermatitis (“spot-ons”), foreign bodies (cactus tines, etc.) and idiopathy. We must never forget that cutaneous adverse drug reactions can mimic all of these reaction patterns.

In addition to a thorough history and physical examination, laboratory evaluations (skin scrapings, tapings, combings, fecal flotations, trichography, cytology, Wood’s lamp, fungal culture, skin biopsies), response to treatment (parasiticides), and avoidance (drugs, contactants, environmental niches) are often indicated to rule out nonallergic disorders. Only then should investigations that pursue allergies (novel diets, serological and intradermal “allergy” tests) be undertaken.

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ALLERGY TESTING IN CATS – IS IT WORTH IT?

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The diagnosis of feline atopic syndrome (FAS) is based on the exclusion of other pruritic
diseases, such as ectoparasites and bacterial and fungal infections, as well as an evaluation of the
role of flea and food allergens as flare factors; compatible clinical signs and on a positive
response to appropriate therapy. Allergy testing can be performed to select the allergens for an
immunotherapy regime and to implement allergen avoidance measures.

Two methods of allergy testing are routinely available for the further investigation of FAS.
Intradermal allergy test (IDT) and in-vitro measurement of allergen-specific IgE (IgE serology).
Negative and positive results can be obtained with either test in clinically normal cats and cats
with other skin diseases. The test results are only meaningful if the cat has clinical signs
consistent with FAS and all other pruritic diseases have been ruled out. Allergy testing is
indicated in cats with history and clinical signs consistent with FAS in which establishment of a
definitive cause is desired, or allergen-specific immunotherapy is being considered as a potential
treatment. It should only be performed after other pruritic diseases have been ruled out or
controlled (ectoparasites, dermatophytes, pyoderma, Malassezia dermatitis, food and flea
allergy). They are useful tests if owners wish to consider immunotherapy as a management
strategy for the cat with chronic allergic dermatitis. There is a great deal of controversy over
which is the more accurate, intradermal allergy testing or serum tests, or whether they are of
value.

Intradermal allergy test
The IDT is a method for demonstrating the presence of hypersensitivity to various environmental
allergens based on skin reactivity. IDT is usually performed at referral centers or in practices in
which there is a clinician with a specific interest in dermatology. The selection of allergens and
interpretation of IDT requires specialized advice and training. Allergen solutions are also
expensive and it would not be cost effective to offer this service unless one or two tests per week
were being performed. Practitioners interested in performing this procedure should study for a
further qualification in the discipline or undertake residency training.

Most veterinary dermatologists test for reactivity against the following antigens:
  • House dust mite and storage mite antigens (Dermatophagoides farinae, 
    Dermatophagoides pteronyssinus, Acarus siro, Tyrophagus putrescentiae)
  • Insect body parts/fecal elements (cockroach, moth, ant, houseflies)
  • Pollens (from trees, weeds and grasses)
  • Molds (from the household or from crops)

The inclusion of regional allergens (pollens) in the testing kit is based on knowledge of the plants
in a particular geographical location. Skin test reactions will vary depending on the location of
the cat. In one Australian study evaluating cats from Sydney, strong reactions were recorded in
68% of cats tested with pollens (grass, weed and/or tree; 61%), followed by insects (46%), including flea (29%) and, less frequently, dust mites [25%; *D. farinae* (18%); *D. pteronyssinus* (14%)] and molds (11%) (Ravens 2014). The IDT is interpreted by correlating the positive reactions with the cat’s history. Clinically relevant reactions can then be used to choose allergens for specific immunotherapy.

IDT is considered traditionally difficult to perform and interpret in the cat. Some authors suggest that cat skin is thinner, finer and tougher than dog skin and that test reactions can be subtle and can more difficult to interpret than skin reactions in dogs. Challenges include poor and inconsistent wheal formation, an inability to test reactions due to rapid disappearance of the injected allergen solution, the use of non-standardized concentration of allergens and the influence of patient stress. The author is of the view that several strategies can be employed to address these challenges and increase the diagnostic value of the IDT in cats.

The concentration of allergens used for IDT of dogs and cats is a subject of controversy in veterinary dermatology. Current recommendations for test concentrations of plant pollen allergens range from 1000 protein nitrogen units (PNU)/ml to 8000PNU/ml for dogs and until recently there were no specific recommendations published for cats. A concentration of 1000 PNU/ml has been typically used for grass, weed and tree pollens when testing cats and there is no evidence to support that these allergen concentrations accurately identify all cats with FAS.

The irritant threshold concentration (ITC) of any allergen is typically determined by evaluating the lower concentration at which at least 10% of normal patients react. Very few allergens have had their ITC determined for IDT in cats. One study reported that the ITC of flea antigen was 1:750 w/v and below 1000PNU/ml for *Dermatophagoides farinae, Dermatophagoides pteronyssinus*, housefly, mosquito and moth in normal cats but the exact irritant concentration was not determined. The same study failed to identify the ITC in 41 of 48 plant allergens tested. All ITC’s were above 4000PNU/ml which was the highest concentration of allergen tested (Austel 2006). Our group at the Animal Dermatology Clinic-Perth tested 16 common grass, weed and tree pollen allergens at 8000 PNU/ml in healthy, non-allergic cats and used serial dilutions from 4000PNU/ml to 6000PNU/ml for any allergen that had a positive reaction at 8000PNU/ml. This study confirmed that the ITC for *Cynodon dactylon* (Bermuda grass) and *Schinus* spp. (pepper trees) was between 6000 and 8000 PNU/ml and the ITC of all other allergens tested was >8000PNU/ml. These results strongly suggest that the practice of using a test concentration of 1000PNU/ml for plant pollen allergens for IDT cats with potential FAS may not be valid (Scholz 2017). We currently perform all IDT in cats using 8000PNU/ml (except *Cynodon dactylon* and *Schinus* spp).

Stressors including transportation, physical examination and procedures varying from venipuncture to IDT can lead to an increased concentration of serum cortisol in cats. A previous study concluded that increases in cortisol, corticotropin and alpha-melanocyte stimulating hormone after handling and performing IDT in cats may be a reasonable explanation for weak skin test reactions in cats as cortisol suppresses, secretion and the action of inflammatory mediators. A recent study evaluating a single administration of gabapentin at 25 to 30mg/kg two hours prior to the veterinary visit did not significantly decrease cortisol concentrations, and therefore it is unlikely that pre-appointment gabapentin would improve interpretation of IDT in
cats that have weak reactions due to stress (Hudec 2019). However, the administration of gabapentin did not alter IDT reactions and gabapentin is a safe and effective option to decrease stress-induced outward responses in cats.

**Intradermal testing technique**
The procedure is performed with the cat under sedation in lateral recumbency. Medetomidine (Domitor®, Pfizer) at a dose of 70 μg/kg is administered by intramuscular injection. The cat should be housed in a dark, quiet environment until the sedation takes full effect. An intravenous catheter (IV) is then placed into the cephalic vein and a patch of fur clipped from the ventrolateral thorax (about 10cm x 10cm). The injection sites are marked using a black marker pen and approximately 0.05ml of each antigen is injected intradermally along with the positive (histamine solution 0.1mg/ml) and negative (0.9% phosphate buffered saline) control. The reactions are read within 5 to 10 minutes after the injections are administered.

The test reactions are evaluated using a subjective scoring system of 0 to 4+ based on evaluation of erythema, turgidity, size and steepness of the wheal and an objective measurement of the wheal diameter using a ruler. Examination of the reaction sites is made in dim light with an oblique light source to examine the margins of the reaction sites, their size and volume. Erythema in cats with light coloured skin is best visualised under normal lighting. The diameter of the test sites is measured across a round wheal or the smaller dimensions of an oval wheal. A reaction is considered positive when the diameter of the wheal and/or erythema formation is equal to or greater (50 to 75%: 3+ or >75%: 4+) than the mean between the negative and positive control.

Immediately after the test is read, 5mg/kg of 10% fluorescein sodium solution is injected slowly via the IV catheter to facilitate readability and interpretation of the skin test reactions. Test reactions are then interpreted in a dark room with the aid of a Wood’s lamp and both fluorescent intensity and wheal diameter are evaluated.

In a study of 10 normal cats, the measurement of fluorescent wheal diameter was more accurate than the semi-quantitative, subjective assessment of fluorescein intensity (Schleifer 2003). In a more recent study, 10% of cats had positive allergen reactions detected on the basis of fluorescein wheal diameter and intensity staining that would have been otherwise negative if other criteria had been used (Scholz 2017). The administration of IV fluorescein appears to be well tolerated and safe. A case of anaphylaxis subsequent to IV administration of 10% fluorescein has been reported, however an approximate five-fold increase in the dose of 10% fluorescein was administered when compared to the recommended dose rate. In summary, we strongly recommend the use of 5mg/kg of 10% fluorescein sodium solution for IDT in cats to improve readability and interpretation and we have not observed adverse events using this dose rate and protocol. After the testing is completed, sedation is reversed by injecting 0.35mg/kg of atipamezole (Antisedan ® Pfizer) by intramuscular injection. Late phase reactions are not recorded in cats.

**Allergen specific IgE serology test**
A number of allergen-specific serology tests are now available for cats. These tests are attractive because they are easy to perform. The value of serum allergen specific IgE for identifying cats
with FAS is controversial. This is because of poor correlation with the results of skin testing and because feline anti-IgE has questionable specificity in cats. All the studies have failed to demonstrate significant differences in allergen-specific IgE levels in sera from supposed FAS cats and that of normal cats (Taglinger 2005, Bexley 2008). Nevertheless, many dermatologists utilize them to select allergens for immunotherapy in cats with a clinical diagnosis of FAS.

**IgE serology test technique**

In-vitro testing simply requires a blood sample to be taken and sent to an appropriate laboratory. The diluted serum sample is added to a plate containing individual wells coated with specific antigens. The types of antigens tested are similar to those used for IDT, but there are usually less than in a skin test. If there is any IgE in the serum that is specific for a particular antigen, it binds to it. The bound IgE is then detected by adding an enzyme-linked reagent that can bind to IgE. This is either a monoclonal antibody or a receptor for IgE molecules. A substrate is added that changes color when it contacts the enzyme attached to the IgE reagent. The degree of color change is proportional to the amount of IgE that is bound. The color change is measured by an automated reader and the results are reported as a numerical score. The significance of various scores is indicated by the laboratory. The in-vitro test is interpreted by correlating the positive reactions with the patient’s history. Clinically relevant reactions can then be used to choose allergens for specific immunotherapy.

**Preparation of cats for allergy testing**

Before either of the above tests are performed, it is important that the cat is adequately prepared. Clinicians should ensure that other pruritic diseases have been ruled out; the skin is in a suitable condition for skin testing and anti-pruritic drugs have been withdrawn for a suitable period of time.

**Withdrawal times for anti-pruritic drugs before allergy testing**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Intradermal testing</th>
<th>In-vitro testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylprednisolone acetate injection</td>
<td>6-10 weeks</td>
<td>3-5 weeks</td>
</tr>
<tr>
<td>Daily prednisolone</td>
<td>4-6 weeks</td>
<td>0</td>
</tr>
<tr>
<td>Alternate day prednisolone</td>
<td>4-6 weeks</td>
<td>0</td>
</tr>
<tr>
<td>Topical corticosteroids (including ear/eye drops)</td>
<td>1 week</td>
<td>0</td>
</tr>
<tr>
<td>Antihistamines</td>
<td>1 week</td>
<td>0</td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Essential fatty acids</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Oclacitinib</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE:** These times may vary for an individual cat. Treatment with long acting injectable corticosteroids, daily and every other day prednisolone, or cyclosporin for periods longer than three months may require longer withdrawal times.
Intradermal allergy testing versus IgE serology—which test is best?
Veterinary dermatologists are often asked which of the above tests is the best. When answering this question, it is important to remember that the tests are not measuring the same thing. *In vitro* tests measure the amount of allergen-specific IgE that is present in the blood. IDT detects the presence of allergen-specific IgE that is bound to mast cells in the skin. However, IDT also measure mast cell releasability and the response of the skin to inflammatory mediators. IDT, therefore, provide a complete functional assessment of some of the pathways that are required to initiate an allergic reaction in the skin. In contrast, *in vitro* tests only measure one particular point in the pathway. For this reason, most veterinary dermatologists regard IDT as the superior test. If it is not possible for a cat to undergo IDT (e.g., if the practice doesn’t perform it, there is no local referral center, the owner doesn’t want referral), *in vitro* tests can be used as alternative to identify allergens for use in immunotherapy. Performance of both tests at the same time is more informative, although it may be cost prohibitive. Of note, an increase in the efficacy of the chosen immunotherapy based on the combined test results has not been demonstrated.

In summary, as with dogs and human beings, there is no definitive diagnostic test for FAS. The finding of allergen-specific IgE in serum is not diagnostic, since both normal and allergic cats can have detectable serum allergen-specific IgE, supporting the importance of a clinical diagnosis prior to the pursuit of allergy testing. In the author’s opinion, IDT using allergens at a concentration of 6000 to 80000PNU/ml with intravenous fluorescein to enhance readability and interpretation in a calm, well sedated cat appropriately selected for testing is the optimal method of allergy testing cats to support a clinical diagnosis, especially when allergen-specific immunotherapy is being considered for treatment.

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MANAGEMENT OF THE ATOPIC CAT – HAVE WE MOVED AWAY FROM PREDNISOLONE?
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Atopic Dermatitis Syndrome
There have been a variety of terms used to describe cats with allergic skin disease. These have included feline allergic dermatitis (FAD), non-flea, non-food hypersensitivity dermatitis (NFNFHD), feline atopic-like dermatitis, feline cutaneous hypersensitivity or feline hypersensitivity dermatitis and feline allergic dermatitis syndrome (FADS). A consensus from the International Committee on Allergic Diseases of Animals (ICADA) has adopted the term ‘feline atopic syndrome’ to replace all the previous names and to be used from this point forward. This syndrome would specifically exclude parasitic causes, but would include environmental allergen causes, some manifestations of asthma and some manifestations of food reactions. Some food-related disease or respiratory disease occurs outside of the feline atopic syndrome. This syndrome is becoming more commonly recognized in the cat. The resulting pruritus and lesions cannot be differentiated visually from many other diseases (miliary dermatitis presentations, eosinophilic granuloma and feline symmetrical alopecia) and may occur in conjunction with other allergies. Feline atopic syndrome is a type I, IgE-mediated allergic skin disease caused by environmental allergens. It should be considered in any seasonally pruritic patient, but it can also present with year-round signs. In some cases, concurrent asthma or upper airway disease can also be seen.

There is no specific diagnostic test for feline atopic syndrome. It is a clinical diagnosis based on compatible clinical signs and exclusion of other conditions that could cause similar findings. A diagnostic criterion has been established using the concept of exclusion and pattern and lesion evaluation (see below—diagnostic criteria). A logical stepwise approach is required and owners should be made aware that it might take weeks to months to reach a confirmed diagnosis based on the following process:

1. Compatible history and clinical signs (age of onset, lesion distribution). Rule out/treat parasites with flea control, skin scrapes, coat brushings +/- trichograms to look for fleas and mites. Trial therapy may also be necessary to rule out Notoedres and other ectoparasites.
2. Cats with non-seasonal pruritus should undergo a six- to eight-week elimination diet.
3. Rule out/treat infections. Cytology (tape strips/impression smears) to identify yeast (Malassezia) and bacterial infections and fungal culture to rule out dermatophyte infection.

If pruritus persists once adverse food reactions, parasites and infections have been excluded, then feline atopic syndrome is likely.

Criteria set for the diagnosis of feline non-flea hypersensitivity dermatitis (NFHD) feline atopic syndrome

1. Presence of pruritus at onset
2. Presence of at least two body sites affected
3. Presence of at least two of the four following clinical patterns:
   - Symmetrical alopecia
   - Miliary dermatitis
   - Eosinophilic dermatitis
   - Head and neck erosions/ulcerations
4. Presence of any lesion on the lips
5. Presence of erosions or ulcerations on the chin or neck
6. Absence of lesions on the rump
7. Absence of non-symmetrical alopecia on the rump or tail
8. Absence of nodules or tumors

Fulfillment of five of the eight criteria gives a sensitivity of 75% and a specificity of 76% for the diagnosis of NFHD

**Criteria set for the diagnosis of feline NFHD after exclusion of flea HD**


1. Presence of pruritus at onset
2. Presence of at least two of the following classical clinical reaction patterns:
   - Symmetrical alopecia
   - Miliary dermatitis
   - Eosinophilic dermatitis
   - Head and neck erosions/ulcerations
3. Presence of at least two sites affected
4. Presence of miliary dermatitis as a dominant pattern
5. Presence of eosinophilic dermatitis or symmetrical alopecia or erosions/ulcerations on the head, face, lips, ears or neck
6. Presence of non-symmetrical alopecia on the rump, tail or hind limbs
7. Presence of symmetrical alopecia on the abdomen
8. Absence of erosions/ulcerations on the forelimbs
9. Absence of lesions on the sternum or axilla
10. Absence of nodules or tumors

Fulfillment of six of these 10 criteria gives a sensitivity of 90% and a specificity of 83% for the diagnosis of NFHD.

Although intradermal testing (IDT) is considered the preferred allergy test in the canine, it is more controversial in the feline and reactions are more difficult to read. There are reports
demonstrating the benefits of highlighting positive reactions of IDT in cats when fluorescein was administered intravenously and fluorescent wheals were evaluated under ultraviolet radiation (Wood’s lamp). Because of the difficulty to perform and read IDT in the cat and due to the success seen with some of the serum in vitro testing (SIVT) companies, the author will utilize SIVT as an option or in combination with IDT. As in the dog, SIVT in the cat should be used for allergen selection for allergen specific immunotherapy (ASIT) and not for diagnosing feline atopic syndrome. The results of allergy testing can also be used for avoidance or control of some environmental allergens. Optimal glucocorticoid withdrawal times should be followed, similar to those recommended in the canine for the best results.

Although newer options for glucocorticoids are being explored in the cat, they still remain one of the mainstay therapies to control clinical signs in the atopic cat. Glucocorticoids are a very successful mode of therapy in feline atopic dermatitis syndrome, but care should be used when administering glucocorticoids in cats. Oral methylprednisolone at 1-1.5 mg/kg q24h initially is often needed with tapering to 0.5 mg/kg q48h after 7 to 10 days. Methylprednisolone acetate (Depomedrol, Zoetis) can also be used at 4 mg/kg IM. Occasionally other oral glucocorticoids are used. The author will reach for oral triamcinolone and dexamethasone in more refractory cases. The efficacy of antihistamines in the cat are not overly impressive, but occasional responses can be seen in milder forms of the disease with chlorpheniramine at 2-4 mg q12h, amitriptyline 10 mg q12-24h, loratadine 5mg q12-24h, clemastine 0.67 mg q12-24h, cetirizine 5-10 mg q24h and cyproheptadine 1-2 mg q12-24h.

One of the more effective treatment options for feline atopic dermatitis syndrome is oral cyclosporine (Atopica® for Cats, Elanco). It is dosed at 5-10mg/kg q24h. Cyclosporine has also been used in all forms of eosinophilic granuloma complex and cases of feline asthma; conditions seen in the atopic dermatitis syndrome. Some gastrointestinal side effects and anorexia are seen in a limited number of cases and avoiding its use in patients with viral infections is recommended. Monitoring for renal side effects is recommended. However, the most common side effect is vomiting in a limited number of cases. An occasional case may also be more prone to the development of upper respiratory viruses. Screening prior to and during therapy for toxoplasmosis is also recommended, as there are some concerns related to disseminated infections in cats treated with cyclosporine.

Oclacitinib (Apoquel®, Zoetis) is currently being evaluated in the cat and some safety profile data has been accumulated by Zoetis. A recent controlled study showed good safety data with no side effects at dosages of 1-2 mg/kg q12hrs in a 28-day treatment period. There are now a number of papers published using oclacitinib in the cat. In an experimental model using IL-31–induced pruritus in cats, oclacitinib given at 0.4 mg/kg or 1 mg/kg one hour before administration of IL-31 reduced pruritus in 63% and 62% of the test animals, respectively. A report evaluated the use of oclacitinib for nonflea-nonfood-induced hypersensitivity dermatitis (NFAFHD) in cats using a dose of 0.4-0.6 mg/kg orally q12hrs for 14 days, then q 24hrs for an additional 14 days. Clinical lesions were evaluated with the Scoring Feline Allergic Dermatitis (SCORFAD) system and pruritus was evaluated with a 10-cm-long visual analog scale (VAS) before and at the end of the study. There was good improvement in SCORFAD and VAS pruritus scores in five of 12 cases, while the other cats were unchanged, deteriorated or withdrew from the study due to treatment failure. A higher dose of 1mg/kg given twice daily for 31 days.
was reported to provide a good clinical response in a case of feline cutaneous mastocytosis with no adverse effects observed.

In cats with experimental asthma, oclacitinib at 0.5 mg/kg or 1 mg/kg twice daily for 28 days significantly suppressed airway inflammation and adverse clinical signs were not observed. Oclacitinib also was successfully used in a case report of feline idiopathic ulcerative dermatitis at dosages of 1.5–2mg/kg/day. In yet another report, dosage ranging from 0.8–1.3mg/kg twice daily was effective in cats with NFNFHD. The author has had success using oclacitinib for the management of pruritus in a limited number of cats with atopic dermatitis syndrome. A minimum dose of 1mg/kg q 12hrs for induction is a typical starting point and, depending upon response, may be reduced to 0.5 mg/kg q 12hrs after the first 2 to 4 weeks.

The caninized monoclonal antibody directed against interleukin-31, lokivetmab (Cytopoint®, Zoetis), for the management of canine AD is not recommended for cats as it is not a feline-based product and could create adverse reactions to the foreign protein; efficacy would be questionable. A feline monoclonal antibody is currently being evaluated but no published information is currently available on its success or potential release.

Allergen Specific Immunotherapy (ASIT) is a commonly used mainstay therapy in many cats with atopic dermatitis syndrome. Although double-blinded placebo-controlled studies on the efficacy of ASIT are still lacking in the cat, open studies showed success rates that varied from 45% to 100%. ASIT may also represent the most appropriate long-term control method for a cat with non-seasonal atopic disease. The success of ASIT will depend on the accuracy of the diagnosis, the formulation of the vaccine (selection of the allergens) and the realization that each program needs to be tailored to the specific needs of the patient, with regard to frequency of injections and number of allergens used. In addition, ASIT requires compliant owners and cooperative patients. The recommended induction protocol of ASIT is similar in cat as in the dog. However, the author prefers to use a lower maintenance dose of 0.5 ml every 1 to 2 weeks (rather than common canine protocols of 1 ml every 10 days to 3 weeks). Again, interval between injections may be decreased or increased according to the response obtained. Some cats with severe pruritus may also require the administration of symptomatic therapy during the induction phase of ASIT, while waiting for the benefits of ASIT to become clinically apparent.

An alternative option to conventional injection immunotherapy is rush immunotherapy. While still administering immunotherapy via subcutaneous injection, this method eliminates the induction phase of the immunotherapy protocol. Gradually increasing amounts of allergen are administered over several hours as opposed to several weeks until the maintenance dose is reached. It appears safe in limited studies and anecdotal reports and allows owners to go to maintenance intervals immediately. Another option to conventional or rush injectable immunotherapy, is sublingual ASIT which utilizes small drops of allergen extract that are given via the oral mucosa. No controlled studies are available comparing the efficacy of oral vs subcutaneous ASIT, but anecdotally it appears effective in some cases. The author still prefers subcutaneous ASIT in cats as giving injections in most cats is often easier than giving oral medications and the interval between administration is longer compared to daily to twice daily sublingual therapy.
Many cats with feline atopic syndrome are prone to secondary pyoderma and Malassezia dermatitis and otitis. These secondary infections can aggravate the pruritus and need to be addressed with appropriate antimicrobials.

**Adverse Food Reactions**

Adverse food reactions are uncommon as a sole entity in the cat but may occur more frequently as combination hypersensitivity with concurrent atopic dermatitis syndrome. As a result of this, most cats with compatible signs of atopic dermatitis syndrome should have elimination diet trials performed. Adverse food reactions contribute to some of the cases of eosinophilic granuloma complex or feline symmetrical alopecia. No age predilections have been reported. One report suggests males may be at a slight predilection and two studies suggest Siamese cats may be at increased risk. Most food allergic cats have a history of year-round pruritus. Although some cats are refractory to or require higher than usual dosages of glucocorticoids, many cats commonly respond to anti-inflammatory dosages. Adverse food reactions typically present with pruritus, excoriations and alopecia of the head, face, preauricular areas, axilla or total body. Some pruritic cats may lack obvious lesions. In addition to the scaling, crusting and pruritus, the clinical signs may include urticaria, angioedema or otitis externa. Concurrent gastrointestinal signs can also be seen.

The diagnosis is made by placing the cat on an elimination diet, a food source that the cat has not previously received. Switching from one commercial food to another is not acceptable since many of these contain similar ingredients. The author prefers an eight-week course consisting of commercial novel or hydrolyzed diet that has not been previously fed. Examples of limited protein based commercial diets or hydrolyzed diets include: Royal Canin (ie rabbit and green pea), Rayne Clinical Nutrition (Rabbit or Kangaroo), Hills (Feline ZD low allergen, a hydrolyzed chicken and chicken liver diet), Royal Canin Hydrolyzed Diet (hydrolyzed soy and poultry liver, rice, beet) and Purina Feline CMN-HA (hydrolyzed soy and rice as starch). Limitations of these diets can include anorexia and on occasion gastrointestinal problems, such as vomiting and diarrhea. At the end of eight weeks, the cat is re-challenged with the original diet and observed for exacerbation of clinical signs. Some of the above-mentioned diets can be used as maintenance diets as well. If the cat is severely pruritic at the time of the elimination diet, an Elizabethan collar or a short course of glucocorticoids may be used to break the "itch-scratch" cycle. Concurrent antibiotics are often also needed.

**Flea Allergic Dermatitis**

Flea allergy can be a concurrent issue with atopic dermatitis syndrome and overlap the clinical patterns and intensify symptoms. As a result of this it is important in flea endemic areas to maintain good flea control as part of managing cats with atopic dermatitis syndrome. Choice of which flea control products to utilize is often a reflection of personal preference, client and specific case factors. In the last several years, there has been a variety of new flea products brought to market, all increasing the options for more complete flea control. Traditional products, such as imidacloprid, fipronil, nitenpyram and lufenuron have commonly been replaced by practitioners with newer options, although these products can still be effective in certain households and geographical regions. Currently the author is using a variety of the newer products, including five topical products indoxacarb (Activyl, Merck), dinotefuran (Vectra, Ceva), spinetoram (Cherstin, Elanco), fluralaner (Bravecto for cats topical solution-Merck),
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PYODERMA IN CATS – IS IT REALLY A PROBLEM?

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Although “pyoderma” literally means “pus in the skin”, the term is traditionally used to indicate bacterial infection. In order to limit the scope of this discussion, I am going to focus on bacterial folliculitis and furunculosis.

Until recently, bacterial folliculitis and furunculosis were considered to be rare, with only limited reports available in the literature. However, bacterial folliculitis and furunculosis accounted for 13% of all patients and 10% of all dermatologic diagnoses in cats examined by a university dermatology service. Similarly, superficial bacterial pyoderma accounted for 20% of the total feline patients presented to a dermatology referral service.

The causative bacteria in feline folliculitis and furunculosis include: Staphylococcus pseudintermedius, S. aureus, and S. simulans. The disorders frequently occur in association with trauma, drug- or disease-induced immunosuppression, ectoparasitism, and allergies. In one study, bacterial folliculitis and furunculosis were secondary to another dermatologic disease – especially allergies and feline acne – in 71% of the cases. In another report, 15% of the cats with atopic dermatitis had concurrent bacterial folliculitis and/or furunculosis.

The common lesions seen with feline bacterial folliculitis and furunculosis include papules, crusts, annular erosions and/or ulcers, and alopecia. Pustules and epidermal collarettes are seen infrequently. Pruritus is common, especially in cats with associated allergies (atopic dermatitis, food allergy, flea-bite allergy). Lesions may be present at any cutaneous site, and their location often mirrors where the underlying disease is active (especially face, neck, head, neck, dorsum).

The presence of bacterial infection is confirmed by cytologic examination (suppurative-to-pyogranulomatous inflammation with degenerate neutrophils and phagocytosed bacteria). Bacterial culture and susceptibility testing are not usually performed, unless antibiotic resistance is suspected. Recognition and control of an associated disorder is often the key to preventing recurrent infection.

First-line systemic antibiotics of choice include amoxicillin clavulante, cefpodoxime, cefovicin, and clindamycin. Topical antimicrobials – other than those used to treat infected feline acne – are not usually well-tolerated (by patient and/or owner). In severely pruritic cats, short-term systemic glucocorticoid (prednisolone, dexamethasone) administration may be required for the infection and/or underlying disorder is under control.

Selected References
DERMATOSES OF THE FACE, HEAD AND NECK – HOW OFTEN IS IT FOOD ALLERGY?

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Introduction
A variety of feline dermatoses may affect the face, head and neck, with allergies the most common worldwide, followed by a range of infections with variable geographical distributions, and occasional auto-immune, metabolic or neoplastic diseases. Diagnostically, the important first task for clinicians is to distinguish allergies from the other causes, for which some historical and clinical clues can be valuable. Assuming allergies are most likely, the second task is determining the specific allergy, with atopic dermatitis, food allergy and flea allergy the major considerations. Allergen avoidance generally provides resolution of signs for food and flea allergy, while atopic dermatitis often requires a life-long treatment plan.

QUESTION ONE: Is there Infection?
Infectious causes of face, head, and neck dermatitis may present with a variety of lesions.

Alopecia, Erythema and Scaling to Erosive/Ulcerative and Crusting Presentations
Although allergies are the most likely causes of this presentation, particularly if associated with pruritus, some infections are important considerations.

Dermatophytosis is comparatively common in cats and an important consideration in young, long-haired or recently adopted cats, or those from multi-pet households. It is unlikely in healthy adult cats without a prior history of dermatophytosis and without known contagion risks. Alopecia is usually a feature but varies from characteristic well-demarcated lesions to more subtle focal or diffuse alopecia. Pruritus is often absent to mild and rarely severe.

Demodicosis due to D. cati (classic follicular mite) is associated with underlying immuno-suppression from disease or drug therapies. Alopecia also features; it is often localised and well-demarcated but may be generalised. Pruritus may be mild or absent. In contrast, D. gatoi (short-bodied surface-keratin mite) can occur in healthy cats and appears as pruritus and self-trauma lesions similar to allergic presentations. It is generally rare, but more common in some countries.

Sporadic Infections. Otodectes infestation is a rare cause of pruritic dermatitis of the pre-auricular areas and adjacent skin, in addition to otitis. Lice or Cheyletiella rarely produce regional to generalised scaling with patchy alopecia, with mild to absent pruritus. Herpes viral dermatitis is also rare, appearing as ulcerated discrete facial lesions, with or without pruritus.

Regional Infections. Leishmaniasis is endemic in parts of Europe (Mediterranean), and Central or Southern America; feline infections are rare and often asymptomatic. Ulcers/crusting, alopecia or papules/nodules may occur, most typically on the face, head or neck, together with systemic signs (malaise, lymphadenomegaly, fever). Sporotrichosis occurs sporadically worldwide, but mainly in tropical or subtropical areas, especially Central or Southern America. It may occasionally present with subtle erythema, alopecia, and scaling, but is more typically nodular.
Nodules With or Without Ulcerative/Crusting Lesions
A range of pathogens, particularly traumatically implanted environmental saprophytes, cause sporadic nodular infections on the face, head and/or neck. They include *Mycobacteria*, *Cryptococcus*, and a variety of pigmented or non-pigmented fungi. *Sporotrichosis* most commonly appears as papules or nodules that ulcerate and crust, most typically on the head. *Leishmaniasis* will sometimes present with nodules. Nodular presentations tend to be less diagnostically challenging, with cytology and/or histopathology often diagnostic. Some infections remain localised to the skin and others have the propensity to disseminate.

**History clues that make infectious dermatoses more likely:**
- Breed: long-haired cats, especially Persian breed (dermatophytosis)
- Age: young (<6 months) or aged/immune-compromised
- Geographical region: Europe, Central South America (leishmaniasis; sporotrichosis); USA, UK, Europe (*D. gatoi*)
- Potential exposure: multiple pet household, new kitten, recently rescued cats, cats on immunosuppressive therapies (including for symptomatic treatment of pruritus)
- Zoonosis: skin lesions in family members (dermatophytosis)
- Pattern of itch: persistent rather than intermittently flaring
- Degree of itch: milder, with skin lesions dominating
- Response to treatment: lack of good response to glucocorticoid therapy

**Clinical clues consistent with infectious dermatoses:**
- Lesion types: lesions less suggestive of allergies include well-demarcated complete alopecia or alopecia not readily explained by self-trauma (dermatophytosis), prominent scaling with patchy alopecia (mites or lice), discrete ulcers not readily explained by self-trauma (herpes dermatitis), and nodules (deep infections)
- Lesion sites: pinnae (dermatophytosis), pre-auricular (otodectes)

**Diagnostics to confirm or exclude infectious dermatoses:**
Relevant tests depend on the likely differentials for each cat based on specific history and physical exam findings, and include:
- Close physical examination: Lice
- Cytology
  - Tape Impressions: *Cheyletiella*, Dermatophytosis (appear more sensitive than trichograms as they collect heavily infected small broken hair fragments, that are easily lost with hair plucks)
  - Scrapings: superficial (*D. gatoi*, *Otodectes*) or deep (*D. cati*)
  - Ear swab or curette sample in paraffin oil: *Otodectes*
  - FNA (nodules): Deep infections except *NOT* if suspect Sporotrichosis (zoonotic risk)
- Wood’s Lamp: some world variation, but approximately 50% *M. canis* infections positive
- Dermatophyte culture (from hairs and superficial scrapings from periphery of lesions, or toothbrush sampling for subtle lesions): may get false negatives, false positives (incorrect interpretation in-house tests, occasional transient carriage in contaminated environments)
- Skin biopsies: Dermatophytosis (when diagnosis is uncertain; is faster and more definitive than fungal culture alone); Deep Infections
• Real-time PCR of hairs/scale and/or tissue biopsy (variably available): Dermatophytosis, Leishmaniasis, Mycobacteria, Other infections. A positive PCR alone does not confirm infection (previous resolved infection, transient carriage), and a negative makes infection less likely but sensitivity is dependent on sample collection.

QUESTION TWO: Is this an Autoimmune Disease, Sterile Inflammatory Process or Neoplasia?
This group of diseases in cats is rare, but will mimic infectious or allergic causes of face, head or neck dermatoses. There are typically prominent lesions, with minimal or no pruritus, and/or lesions not readily explained by self-trauma from pruritus. Lesions persist at the same sites, with or without extension to new sites.

Crusted to Erosive/Ulcerative Presentations
_Pemphigus foliaceus_ is the most common auto-immune dermatosis in the cat. It presents with crusting to erosive skin lesions, and occasional pustules, most typically in middle-aged cats. The face/head or neck are commonly affected (~90% of cats), including the pinnae (70-90%). Pruritus has been reported in 30-60% of cats and systemic signs in 30-70% (malaise, lethargy, pyrexia). Cytology and/or biopsy confirms acantholysis with the absence of bacteria or fungi.

_Solar or Actinic Dermatitis_ is common on non-pigmented sparsely or non-haired areas in cats with outdoor exposure in countries with high UV indices. Lesions are most common on the pinnae, nasal planum, and periocular or preauricular areas, and include erythema, scaling and scared/aged skin. Pruritus is typically absent. Indoor cats may also be affected as UVA rays penetrate window glass, even though UVB rays are blocked. Solar dermatitis commonly progresses to solar keratosis and squamous cell carcinoma over time.

_SCC in-situ_ is well recognised in middle-aged to older cats of varying breeds as localised single or multifocal pigmented scaly plaques, that slowly progress to ulcerative and crusted plaques or nodules. The face, head or neck are commonly affected, and lesions are typically asymmetrical and may be pruritic. At least some forms are associated with papillomavirus infection, including an aggressive severe form in Devon Rex cats, with progressive development of extensive lesions from a young age, and potential internal metastasis of papillomavirus-associated SCC.

_Ulcerative neck dermatitis_ is described in cats as crusted, non-healing ulceration most typically on the dorsal neck or between the scapulae. Severe scratching is typical, with persistent severe self-trauma often associated with poor healing and/or repeated relapse. There are multiple potential underlying causes, including allergies, stress/anxiety and neuropathic itch. Sustained resolution after glucocorticoid therapy or surgical resection in some cats suggests some transient causes. Underlying allergies appears most frequent, and secondary bacterial infection may be important. Diagnostic evaluation requires consideration of multiple causes of pruritus, especially hypersensitivities. Histopathology may reveal linear sub-epidermal fibrosis, consistent with non-specific chronic trauma. Lesions are often refractory to glucocorticoids and/or antibiotics. Restricting self-trauma (neck bandaging, body suits, bandaging of hind feet) and resolving any secondary bacterial infection are important first steps. Topical antibiotics (e.g. mupirocin,
sodium fusidate, silver sulphadiazine) may be more effective for infection than systemic antibiotics. Management of any underlying disease is important to limit recurrence.

**Papular To Nodular Presentations**

*Urticaria Pigmentosa* has been described as a papular to macular dermatitis in cats characterized histologically by a mastocytic +/- eosinophilic dermatitis. However, similar changes occur with allergies, and also dermatophytosis, so it may be a reaction pattern rather than a distinct disease, with multiple causes possible. It is described in young sparsely haired feline breeds (Sphinx, Devon rex), and the Himalayan. Many cats are pruritic. Typical lesions include erythematous papules to macules, with or without crusting or hyper-pigmentation, occasionally in striking linear patterns. Lesions may affect the neck, head, limbs and/or ventrolateral abdomen.

*Xanthomas.* Multiple pale yellow to pink plaques to small nodules occur rarely in cats associated with abnormalities in lipid metabolism, including hereditary hyperlipidaemia and diabetes mellitus. Lesions are common on the head and may be pruritic. Characteristic large xanthomatous macrophages and free lipid deposits are evident histologically.

*Sterile Pyogranuloma* is reported rarely in cats, appearing as erythematous to violaceous plaques or papules or, less often, as discrete nodules affecting the head most frequently (face, pre-auricular, pinnae). The etiology is unknown. Histopathology reveals discrete pyogranulomatous to granulomatous nodular inflammation, with an absence of infectious agents.

*Neoplasia.* Neoplastic nodular skin lesions will be visually indistinguishable from infectious and inflammatory nodules and require cytology and/or histopathology for confirmation. Although skin neoplasia is more frequently malignant in cats than dogs, many forms are locally aggressive with a low risk of metastasis. Aggressive forms with a higher risk of metastasis include some mast cell tumours, malignant melanoma, and histiocytic sarcomas.

**Alopecic and/or Scaling Presentations**

*Sebaceous adenitis* is reported rarely in cats and characterised by progressive alopecia and scaling, with or without follicular casts. It typically begins on the face and neck, and often progresses to be generalised. Adherent brown-black scale to light crusting is frequent on the periocular area and may also occur on the nasal folds or perioral regions. Some cats may have secondary bacterial pyoderma or otitis externa. Pruritus is typically absent, but occasionally moderate to severe. Skin biopsies are required for diagnosis, revealing an absent of sebaceous glands or a nodular lymphocytic to histiocytic unilateral perifollicular infiltrate targeting the site of sebaceous glands and/or surrounding sebaceous gland remnants. Unlike the canine disease, there is often concurrent mural lymphocytic folliculitis, focused at the region of the entrance of the sebaceous duct. Similar changes may occur in some other feline diseases including thymoma-associated and non-thymoma associated exfoliative dermatitis.

*Mural Folliculitis* is a histological reaction pattern that can occur with a range of feline inflammatory dermatoses, including infections (demodicosis, dermatophytosis), allergies (atopic dermatitis, flea allergy, food allergy), sterile inflammation (alopecia areata, pseudopelade, sebaceous adenitis), and systemic causes (adverse drug reactions, thymoma-associated exfoliative dermatitis). Inflammatory cells target the epithelium of the hair follicle outer root
sheath, most commonly in the infundibular region, and less frequently the isthmus or bulbar regions. Lymphocytic mural folliculitis is the most common form and has been associated with hypersensitivities (~67%) more often than non-allergic dermatoses (33%). Pyogranulomatous mural folliculitis (predominantly affecting the isthmus region) is reported in association with prominent follicular mucinosis in cats with generalised alopecia (most severe over the face, head, neck and shoulders in some cats), with concurrent thickened and swollen facial skin, and variable scaling, crusting and hyperpigmentation. Progression to epitheliotropic lymphoma is reported in some cats, similar to human follicular mucinosis. Concurrent FIV infection has been confirmed in some cats. A biopsy report of mural folliculitis raises the potential of multiple underlying causes and evaluation for multiple potential underlying causes is often indicated. Screening for development of epitheliotropic lymphoma is important with follicular mucinosis.

Non-Thymoma and Thymoma Associated Exfoliative Dermatitis. Severe scaling, often in large white flakes, with a lack of follicular casts and variable alopecia, has been described both with and without thymoma. It is most typically generalised, but may be multifocal, with lesions more severe or occasionally restricted to the head and neck (non-thymoma forms). Pruritus is rare at the onset of lesions but may develop. Systemic signs of lethargy, anorexia and weight loss are typical with concurrent thymoma, but may precede skin changes. Histopathology is characterized by interface epidermal and follicular dermatitis, with or without sebaceous adenitis/absence of sebaceous glands.

Hypereosinophilic Syndrome is rare in cats and characterized by sustained marked peripheral blood and tissue eosinophilia (infiltrates in bone marrow, intestinal tract, lymph nodes, liver, spleen, and sometimes skin). Cats are typically middle-aged (mean 7 years; wide range) with anorexia, weight loss, vomiting, diarrhea, hematochezia and pyrexia. Occasional cats, typically younger, have skin lesions and/or pruritus as the first signs. Skin lesions include extensive areas of alopecia and erythema, with or without multifocal crusted erosions and ulceration, typically involving the head, neck and trunk (ventral and/or dorsal). Diagnosis requires exclusion of other causes of eosinophilia including allergies, parasites, and some auto-immune (e.g., pemphigus group) or neoplastic diseases (e.g., intestinal T-cell lymphoma). Hematology reveals circulating mature eosinophilia (20 to > 50 x 10⁹/L is typical, with normal morphology; 2.7-5.5 x 10⁹/L in some cases, including some with skin lesions). Cytology reveals eosinophilic inflammation. Histopathology reveals extensive superficial to deep interstitial to perivascular dermal eosinophilic infiltrate, with fewer mast cells and/or lymphocytes; not distinguishable from allergic disease. The prognosis is poor, and most cats die or are euthanased. Glucocorticoid therapy is often poorly effective, although higher doses (prednisolone 3 mg/kg twice daily) may be more efficacious. Ciclosporin or imatinib (a tyrosine-kinase inhibitor), with concurrent prednisolone, have been effective in some cases. Optimal management likely involves co-management by internal medicine, oncology and dermatology teams.

**QUESTION THREE: Assuming allergy is likely, which allergy is present?**

Allergies are the most common cause of face, head and neck dermatitis in cats and are likely when pruritus is severe, or when specific clues suggesting infections, auto-immune or rare sterile inflammatory or neoplastic diseases are absent. Diagnostic tests are not always required to exclude non-allergic causes of pruritus: historical and/or clinical findings may be inconsistent with infectious causes (e.g., complete response to glucocorticoid-therapy or intermittently flaring...
pruritus alternating with stages of complete resolution). Screening for secondary bacterial or yeast infection may still be important. When allergy is likely or confirmed, consideration of the specific type of allergy is indicated to enable optimal treatment strategies.

**Historical clues that make allergies more likely:**
- Breed: domestic breeds and some pure breeds at higher risk for atopic dermatitis
- Age-of-onset: young adult cats most typically affected (wide range from months to aged)
- Pattern of itch: recurrent or episodic pruritus strongly suggests allergies except for food allergy alone; constant pruritus is consistent with both allergies and infections
- Degree of itch: severe pruritus is most likely due to allergy +/– secondary bacteria, yeast
- Response to treatment: allergy is typically glucocorticoid-responsive (e.g., prednisolone 1mg/kg once daily x 5-7 days); however, not all cases respond (particularly when complicated by secondary bacterial and/or yeast infections)

**Clinical clues that make allergies more likely:**
- Lesion types: a variety of presentations of feline allergy are recognised:
  - Patchy alopecia, crusting, and excoriations/erosions/ulceration are most common, however, these non-specific lesions do not confirm allergy
  - Eosinophilic granuloma complex: granulomas and plaques suggest allergies; lip ulcers are common with allergies but may occur with other causes of pruritus
- Lesion sites: a wide range of skin regions may be affected with allergies: most classically including the face, head and/or neck for food allergy and atopic dermatitis; the rump, tail, and/or ventral abdomen for flea allergy; lip and/or oral lesions occur in all allergies

**Food Allergy/Adverse Reaction** The risk of food allergy is comparatively low, with a prevalence of 3.4-6% reported in Sydney and New York, compared to 12-14% for atopic dermatitis, and ~4% for flea allergy (New York data only). Dietary trials may be difficult to perform in cats, so screening the history for inconsistent clues is prudent before recommending food trials: pruritus should be perennial (all year round); a history of waxing/waning or episodic pruritus is inconsistent with food allergy alone. Consistent historical and clinical clues include:
- Age-of-onset: mean 4 years (wide range: 3 months to 15 years)
- Pattern of itch: constant (unless concurrent atopic dermatitis or flea allergy)
- Other body signs: diarrhea and/or vomiting most common, affecting up to half of cats; occasional conjunctivitis or hyperactivity reported
- Lesion sites: head/face affected (~50% of cats); ears, ventral abdomen also common

A diagnosis of food allergy/adverse reaction in cats is reliant on elimination dietary trials followed by provocation trials; serum allergen-testing for food allergens is not recommended or validated. A suitable diet for each cat requires a thorough diet history, including brands and flavors of commercial diets. Chicken, beef, pork and fish are common in many commercial diets, and multiple proteins may be present despite the labelled flavor. Beef, fish and chicken are the most frequently identified food allergens for cats. However, the more likely food allergens are influenced by each cats’ previous diet. Elimination diet options are:
- Home-prepared novel protein (appropriate novel proteins are dependent on previous diet)
- Commercial novel-protein (appropriate novel proteins are dependent on previous diet)
  Commercial hydrolyzed: may not reliably exclude food allergy (some cats with food allergy are documented to not tolerate at least some hydrolyzed options)
• Mixed diets: combinations of above (providing some variety can improve compliance)

Important phases of a dietary trial are:
• Elimination phase: 4-6 weeks (>90% of cats achieve clinical remission by 4 weeks)
• Rechallenge phase (smorgasbord): up to 2 weeks (sustained or progressive relapse of signs within 2 weeks suggestive of food allergy)
• Repeat elimination phase: 1-2 weeks (resolution of signs with repeat avoidance confirms food allergy)
• Individual protein challenge: Single proteins for 1-2 weeks at a time (dependent on time to flare for that cat) to identify offending food proteins/formulations

Flea Allergy is an important consideration in all cats living in flea-endemic regions. Consistent historical and clinical clues include:
• Pattern of itch: seasonal (in temperate climates) to non-seasonal; classically, intermittent severe flares interspersed with periods of complete resolution (as for atopic dermatitis)
• Flea control: variable; none to regular, depending on the level of environmental exposure
• Likelihood of exposure: outdoor access, multi-pet households or living in densely populated regions at higher risk; solely indoor cats may still be affected in densely populated, flea-endemic regions
• Lesion sites: rump/tail, ventral abdomen and dorsum +/- neck excoriations, lip ulcers
• Flea presence: fleas and/or flea dirt may be absent (cats are very efficient groomers, and may remove all evidence of low-level flea exposure)

A diagnosis of flea allergy is reliant on response to heightened flea control, with resolution of pruritus expected within 4 to 8 weeks of adulticide therapy using products with a fast speed-of-kill (e.g., sarolaner with selamectin, fluralaner, or imidacloprid). Concurrent environmental treatment with insect growth regulator sprays (e.g., insect surface sprays) may hasten response to 4 weeks and is important in recent outbreak or higher risk scenarios.

Mosquito-bite Allergy is comparatively rare, but often clinically distinct. Consistent historical and clinical clues include:
• Pattern of itch: classically seasonal, with intermittent flares corresponding with times of highest mosquito survival in the environment (warm, moist)
• Lesion types: early/characteristic lesions are tiny clustered papules; excoriations/erosions/ulceration may complicate due to self-trauma
• Lesion sites: restricted to nasal planum/nasal bridge, pinnae, footpads (sites of mosquito feeding and sometimes adjacent self-trauma lesions)

Diagnosis is often readily made due to classical lesions and sites, together with potential mosquito exposure, which collectively are highly suggestive. Lesions resolve completely, with prevention of exposure (e.g., confinement to indoors, prevention of outdoor access at dawn/dusk and/or prevention of sleeping outdoors in high risk scenarios), typically within 2 weeks. Insect repellents may be helpful (e.g., Seresto collars, or topicals [latter often poorly tolerated]).

Atopic Dermatitis (atopic-like syndrome, non-flea non-food allergy) is a life-long allergy requiring effective on-going management plans. Persistent signs or recurrent flares are to be expected and good communication with clients is essential for realistic expectations. Step one is an accurate diagnosis: this does not require tests to exclude all other dermatoses, but rather tests
as indicated to exclude relevant differentials for each individual. Consistent historical and clinical clues include:

- **Breed:** Abyssinian, Himalayan, Persian and Devon Rex appear predisposed
- **Age-of-onset:** Mean 2 years (wide range from 3 months to 15 years; 62% < 3 years)
- **Pattern of itch:** may vary from constant to episodic to seasonal; waxing/waning common
- **Lesion types:** non-specific patchy alopecia, crusting, excoriations/erosions/ulceration are most common (91%); a variety of ‘reaction patterns’ have been described, but mixed patterns are common, lesions are non-specific and all patterns occur with all allergies
- **Lesion sites:** head/face often affected (50-70%); ears, ventral abdomen also common
- **Otitis externa concurrent (16%)**

A diagnosis of atopic dermatitis requires sufficient consistent historical and clinical clues and, where relevant, tests to exclude infections, flea allergy (may be less important with severe face/pinnal lesions, solely indoor cats on flea control and consistent flea control in scenarios of unlikely exposure), and food allergy (unless signs are waxing and waning, intermittent or seasonally recurrent). Food trials may be warranted for perennial fluctuating pruritus to detect concurrent food allergy (but food allergy alone is not consistent with fluctuations in pruritus).

**QUESTION FOUR: Is there secondary bacterial or yeast infection?**

Secondary bacterial and, less commonly, yeast infections are now recognised as common complications of feline dermatoses (10-20% of skin cases at referral), especially allergies. They may create notable pruritus and be responsible for severe flares of pruritus in allergic cats.

Consistent historical and clinical clues include:

- **Pattern and degree of itch:** persistent, and particularly if pruritus is severe
- **Response to treatment:** incomplete response to glucocorticoid therapy
- **Lesion types:** erosions/excoriations, crusting, alopecia and erythema are common, but non-specific; eosinophilic plaques are commonly infected
- **Lesion sites:** the face and neck are among the most commonly affected sites

Skin cytology is usually straightforward for diagnosis, with tape impressions easy and reliable. Bacterial pyoderma is often readily confirmed, with numerous bacterial cocci associated with neutrophils. Malassezia dermatitis is also often readily confirmed, with numerous yeast present. Cytology may be less sensitive for papular (e.g., miliary dermatitis) or nodular (e.g., eosinophilic granuloma) presentations, when histopathology should confirm.

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THE EOSINOPHILIC GRANULOMA COMPLEX – HAS ANYTHING CHANGED?

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The feline eosinophilic granuloma complex (EGC) is a group of lesions that affect the skin, mucocutaneous junctions, oral cavity and combinations of these. It is important to understand that EGC is not a diagnosis, but rather a mucocutaneous reaction pattern with many known associations (Table 1).

The EGC consists of three clinically-distinct lesions. The indolent ulcer ([IU], eosinophilic ulcer, rodent ulcer, lip/labial ulcer). These lesions are unilateral or bilateral, often affecting the upper lip, and neither pruritic nor painful. Histologically the lesions are neither granulomatous nor eosinophilic.

Eosinophilic plaques (EP) are often multiple, raised, extremely pruritic plaques on the bilateral abdomen and medial thighs. Histologically these lesions are characterized by superficial and deep interstitial-to-diffuse eosinophilic dermatitis. Collagen flame figures are occasionally seen. EP are not granulomatous.

The eosinophilic granulomas ([EG], linear granuloma, collagenolytic granuloma) are papular-to-nodular-to-linear plaques, the overlying integument appearing initially normal, and commonly occurring on the lips, chin, caudal thighs, and pawpads. Pruritus varies from absent to marked. Histologically these lesions are characterized by eosinophilic, often palisaded granulomatous dermatitis with numerous multinucleated histiocytic giant cells and collagen flame figures.

Confusion over potentially overlapping histopathological findings in these three clinical lesions has led some to suggest the term “feline eosinophilic dermatitis” be used. I do not find this to be useful, and presume that some of the confusion relates to lesion selection.

In the presentation, the author will review the EGC as concerns clinical associations, various histopathological nuances, the importance of bacterial infection in some of the lesions, and the category of idiopathic EGC.

<table>
<thead>
<tr>
<th>Table 1: Potential Causes of the Feline Eosinophilic Granuloma Complex</th>
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<tr>
<td><strong>Allergic</strong></td>
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<td>Atopic dermatitis</td>
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<td>Food allergy</td>
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<td>Flea-bite allergy</td>
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<td>Mosquito-bite allergy</td>
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<td><strong>Bacterial infection</strong></td>
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<td><strong>Cutaneous adverse drug reaction</strong></td>
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<td><strong>Foreign bodies (cactus tines, insect parts)</strong></td>
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<tr>
<td><strong>Idiopathy (genetic?)</strong></td>
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FELINE OTITIS – IS IT DIFFERENT COMPARED TO DOGS?

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Otitis is defined by inflammation of the ear canal and/or the pinna. Otitis externa (OE) is defined by disease typically involving the external acoustic meatus distal to the tympanic membrane, otitis media (OM) when the tympanic bulla is involved and otitis interna (OI) implies damage to the hearing or vestibular apparatus with neurological signs typically present.

Otitis is typically a multifactorial disease process involving underlying predisposing diseases and subsequent secondary infections. While the basic principles of pathogenesis and treatment of otitis are similar in the cat and dog, there are significant differences in anatomy, etiology and some disease syndromes between the species.

Anatomy

Pinnae. Most cats have upright, open pinnae, but there are genetic mutations leading to pinnal variation. The most common variant is the Scottish Fold cat. Scottish Fold cats have erect pinnae up to four weeks postnatally, but after that the tips of the ears begin to fold rostrally. This folded-ear phenotype is associated with some degree of osteochondrodysplasia of the distal limbs, but has not been reported as an increased risk for otitis. The American curl cat breed has pinnae that are curled back at the pinnal apex. A rare four-eared mutation has also been reported showing a small extra pinna bilaterally. It is associated with reduced globe size and a slightly undershot jaw.

Acquired rostral folding of the ear pinnae is an uncommon disorder associated with iatrogenic hyperadrenocorticism in adult cats. Acquired folding of the ear pinnae may or may not resolve after discontinuation of corticosteroid administration.

Ear Canals and Bulla. The external acoustic meatus (ear canals) in the cat are short, open and hairless compared with most dogs, and may contribute to the comparatively reduced incidence of otitis in this species.

The middle ear anatomy is also significantly different between the dog and cat. The middle ear is an air-filled space located medial to the tympanic membrane and within the middle ear is the bulla septum, which separates the tympanic cavity into two compartments: the dorsolateral epitympanic cavity (pars tympanica) containing the auditory ossicles, osteum of the auditory tube and the eardrum, and the ventromedial tympanic cavity (pars endotympanica). In the dog, the bulla septum is a small, incomplete ridge leaving wide communication between the compartments. In contrast, the bulla septum in the cat is a near complete shelf of bone that extends from the lateral aspect of the tympanic bulla medially to appose the petrous portion of the temporal bone. It allows communication between the two compartments only through two small openings: one between the septum bulla and petrous portion of the temporal bone and the other just lateral to the round window.
This variation in the cat has clinical significance in that:

- otic pathology may be entirely limited to the dorsolateral compartment. However, this is uncommon with disease present in one study in both compartments in 23/24 cats with non-neoplastic OM.\(^1\)
- it may only be possible to effectively flush the dorsolateral compartment of the tympanic bulla. This makes surgical management of the ear necessary in some cases.
- sampling for culture of the disease confined to the ventromedial compartment may not be possible
- topical treatment penetration to the ventromedial compartment may be difficult if desired. Once in the compartment though, drainage of medication from that area may be difficult, leading to persistence of medication.

In contrast to dogs, where the post-ganglionic sympathetic nerves cross near but not through the middle ear, the nerves in cats cross through the bulla making them susceptible to injury resulting in Horner’s syndrome. This is common following middle ear surgery, and less common following myringotomy and subsequent flushing.

**Etiology and Incidence**

While extensive studies are lacking, otitis is reported as less common in cats than dogs, with the disease accounting for around 4% or less of feline veterinary visits. In one study in a university dermatology referral practice, otitis was evident in 13.1% of presented cases\(^2\) but this still represented <1% of total feline visits to the entire university clinic. More recent Australian pet insurance data would support the relatively low incidence of feline otitis, with the disease failing to reach the top ten most frequent claims for cats, in contrast is the most common cause for insurance claims in the dog.

In dogs, multiple studies show that atopic dermatitis and adverse food reactions represent the dominant underlying trigger (~70% of cases) for OE. Conversely, 50-80% of cases of atopic dermatitis and 3-69% of adverse food reactions in dogs will report one or more episodes of OE. Allergies are also a documented trigger for bacterial and/or yeast otitis externa in the cat, but the limited available data suggests this is less common than the dog, occurring in 6.1-17% of cats with allergies / atopic dermatitis.

Studies on the frequency of otitis in stray cats show significant variation depending on the study location (4.6-55.1%) with most common underlying cause being *Otodectes* (2-37%). While pregnancy, an urban habitat and season, were identified as risk factors for otitis and microbial overgrowth, these studies did not further explore the primary causes for otitis in the cats.

The relative frequency of the primary causes of otitis in companion cats has not been well documented compared with the disease in dogs, with the largest study to date Scott et al (2013), though the data presented secondary infections combined with primary aetiologies. To this end a retrospective study was performed at Animal Skin and Ear Specialists (ASES). The records of 100 cases of feline otitis from 1997-2020 were examined and where possible underlying disease triggers recorded (Table 1).
<table>
<thead>
<tr>
<th>Etiology</th>
<th>OE</th>
<th>OE&amp;OM</th>
<th>OM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Very Common</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Confirmed (25) &amp; probable (22) allergies* &amp; atopic dermatitis</td>
<td>47</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Idiopathic inflammation or infection (no confirmed or suspected</td>
<td>12</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>underlying aetiology)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceruminoliths / keratoliths (“plugs”)</td>
<td>10</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><strong>Common</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharyngeal polyps</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Cystadenomatosis</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pemphigus foliaceous</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dirty face syndrome</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Feline immunodeficiency virus</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Uncommon</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteosarcoma of bulla</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Nasal lymphoma</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Trauma</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Inflammatory polyps</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Contact irritant</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Demodicosis (<em>D. cati</em>)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Auricular chondritis</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Urticarial pigmentosa</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Primary failure of self cleaning</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hyperadrenocorticism</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Flea bite hypersensitivity</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total Cases (some cases had &gt;1 diagnosis)</strong></td>
<td>77</td>
<td>9</td>
<td>14</td>
</tr>
</tbody>
</table>

* workup not performed to isolate food allergy vs atopic dermatitis

The results confirm the wide variety of possible aetiologies for feline otitis, but suggest that while OE may be less common in the cat than the dog, when it does occur, non-flea allergies are the dominant cause (61% of cases). This is in broad agreement with Scott et al (2013) who reported allergies the most common cause for *Malassezia* otitis (44.2%) and sterile pruritic allergic otitis the cause of 19.5% of all otitis cases. Ravens et al (2014) also reported 4/45 cats with atopic dermatitis with sterile pruritic otitis externa. Allergies however appear to be a very rare cause for OM, with none found in these cases and only a single case reported in Ravens et al (2014).
Cytology and Microbial Overgrowth in Feline Otitis Externa
As in dogs, both bacterial and yeast overgrowth are common features of feline OE. Cytology and semiquantitative estimation of numbers (Table 2) provides the clinician with a powerful tool to decide if and how to treat the ears, but the presence of organisms in the normal ear can make this a challenge.

Table 2: Criteria for Evaluating the Significance of Organisms on Feline Otic Cytology*

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤2</td>
<td>≤4</td>
</tr>
<tr>
<td>≥12</td>
<td>≥15</td>
</tr>
</tbody>
</table>

When used to differentiate normal from inflamed external ear canals, these figures provided a low sensitivity but a specificity of > or = 95%.

Lipid-dependent and non-lipid dependent *Malassezia* spp have been isolated from the ear canals of both normal cats as well as cats with otitis externa (0-83% of normal cats varying significantly between studies, and 41-95.1% of cats with otitis; including *M. pachydermatitis*, *M. globosa*, *M. furfur*, *M. sympodialis*, *M. obsusa*, *M. slooffiae*, *M. restricta*) though the rate of isolation of *Malassezia* is greater in cats with otitis compared with normal cats. Despite attempts to quantify number of yeast on cytology consistent with a diagnosis of otitis (see Table 2), there still appears to be a subset of cats that carry large numbers of yeast in their ear canals without evidence of otitis. As *Malassezia* may be isolated more frequently from asymptomatic ears in cats with underlying systemic disease (including FIV, and FeLV) compared with normal cats, it has been suggested that the finding of excessive otic organisms in the absence of clinical signs may be indicative of an underlying systemic disease not yet apparent. Unlike the situation in seborrheic Bassett hounds, there is no evidence for an increased prevalence of *Malassezia* otitis in Devon Rex and Sphynx cats prone to *Malassezia* dermatitis.

Similarly, cocci (chiefly *Staphylococcus* and *Streptococcus*, and typically in low numbers) have been reported in 2.5-71% of normal cats, depending on the study. Bacterial overgrowth by cocci is more common in cats with ‘systemic disease’ (including feline urinary syndrome, chronic upper respiratory tract infection and liver, kidney or pancreatic disease) and allergies. Similar to yeast, there is a subset of mainly allergic cats that appear to carry large numbers of bacteria without clinical signs of otitis. In contrast, most studies agree rods are rare in the normal cat and are more likely pathogenic if found.

Hyphal fungi have been cultured and seen on cytological samples from normal cats (up to 70% in one study of stray cats), as well as reported as pathogenic in otitis externa (most commonly *Aspergillus* spp). Immunosuppression (including retroviral infection), otic foreign bodies and previous antibiotics have been reported as risk factors for *Aspergillus* spp. otitis. In the ASES
study above, 5/77 cats (6.5%) with otitis externa showed hyphae on cytology, and in 4/5 of these, fungal hyphae were the only organism evident on cytology. Sporotrichosis has also been reported as a rare cause for feline otitis externa.

Ultimately, the decision to treat or not to treat depends on a combination of the severity of cytological findings combined with clinical signs and a history compatible with OE and, depending on the case, culture results.

Aspects of Non-Neoplastic Otitis Media

Pathogenesis. The pathogenesis of non-neoplastic OM in the cat is not well understood. The finding in the ASES study that 10/13 cases of non-neoplastic OM had no identifiable cause would support this supposition.

OM is proposed to occur primarily as a sequela of upper respiratory tract disease, often as a direct consequence of bacteria from the nasopharynx travelling via the Eustachian tube into the tympanic bulla. This is supported by the findings of bacterial isolates from OM consistent with respiratory pathogens including most commonly Pasteurella multocida (24% in one study), as well as Mycoplasma, Bordatella and rarely Cryptococcus species in addition to the more common staphylococci and streptococci. However only 5/23 cases of OM in the ASES study had a history of upper respiratory tract disease.

Tubal dysfunction and the failure to remove debris from the bulla is also thought to be a necessary precursor for the development of OM. Previous experimental ligation of the Eustachian tube in the cat inevitably resulted in the appearance of sterile fluid and mucoperiosteal hyperplasia in both bulla compartments unless permanent myringotomy was accomplished. This mechanism may explain the finding of culture negative OM in 31-50% of cases, including 7/14 cases of OM in the ASES study above. Allergies, viruses or fungi have also been proposed as possible explanations for the bacterially sterile OM. Tubal dysfunction may also contribute to infection by anaerobic organisms when the eardrum is intact and the Eustachian tube swells, thus sealing these bacteria within the bulla.

Extension of disease from the external ear through the tympanic membrane is considered a less common route of infection in the cat compared with the dog. Less common routes of infection in both cats and dogs include extension via the temporohyoid joint, direct extension via erosion of the tympanic bullae or migration along vascular or neural pathways.

Incidence. There is good evidence in post mortem studies to suggest OM in the absence of OE is clinically underdiagnosed in cats. One study of 50 cats found gross evidence of non-neoplastic middle ear disease in 10/50 cats (14/100 ears) and histological evidence of OM in a further 34/100 ears. Internal ear involvement was present in 11 of 100 ears. The presence of internal ear findings was significantly correlated with more severe OM. Extensive clinical histories were not available in this study but no cats were presented with a history of otitis or neurological signs compatible with OM, though ten cats showed upper respiratory signs, and a further three had no history. A second study found gross lesions compatible with non-neoplastic OM (histopathology was not performed routinely in this study) in 59/3442 (1.7%) cats. Unlike the first study, complete histories were associated with the cases, and critically, only 6/59 cats showed clinical
signs compatible with OM / OI (unilateral vestibular disease in 5/6 and Horner’s syndrome in the remaining cat). These studies suggest that many, if not most, cats with non-neoplastic middle ear disease may not show the expected clinical signs.

Membraneous changes associated with otitis media. In the cat, myringitis (inflammation of the tympanic membrane) is typically reflective of the severity of OM. Early changes include edema and associated opacity. These changes progress to hemorrhage, inflammation, granulation, scarring, vascular ectasia, formation of cholesterol clefts, and bony remodelling of the manubrium of the malleus. These changes are typically similar to, and continuous with, inflammatory changes within the rest of the auricular mucoperiosteum of the middle ear. Rupture of the tympanic membrane, secondary to these inflammatory changes associated with OM, appears to be an infrequent event in the cat, commonly leading to OM without concurrent OE.

During acute OM, the permeability of the membrane of the round window is increased to a variety of macromolecules including some bacterial toxins. This may leave it more vulnerable to ototoxicity. Despite this, morphologic evidence of OI remains relatively uncommon. In response to chronic OM, the membranes of the oval and round windows exhibit inflammatory changes similar to the tympanic membrane and consistent with changes in the auricular mucoperiosteum, including expansion by granulation tissue and inflammatory cells. This results in reduced permeability.

In light of the likely under diagnosis of OM in the cat, clinicians should consider more aggressive use of advanced imaging if there are any abnormalities of the tympanic membrane, even in the absence of supportive clinical signs.

Therapy of OM. Successful therapy of non-neoplastic OM ideally relies on myringotomy if required and thorough cleansing of the bulla followed by appropriate antimicrobial therapy for 10-56 days based on culture and sensitivity results (or positive respiratory bacterial polymerase chain reaction testing in the face of negative culture), with or without 7-10 days of systemic prednisolone post procedure. Studies suggest that 66-73% of cases can be managed successfully in this manner, though there have been reports of cases treated successfully with oral antibiotics alone without bulla flushing. Knowing when to stop oral antimicrobial therapy can be a challenge – continuing until 2 weeks after maximum clinical improvement or until normalization on advanced imaging is ideal.

Surgery (chiefly ventral bulla osteotomy (VBO), less commonly total ear canal ablation) followed by antimicrobial therapy based on culture and sensitivity has a higher success rate (~80-83%) but a higher risk of complications (see below). Cases have been reported that were nonresponsive to medical therapies and resolved with subsequent surgical treatment and vice versa. Some residual neurological signs, if present prior to treatment, are possible even with successful treatment of the infection.

Topical antibiotic treatment of bacterial OM has been reported in human beings using 6% ciprofloxacin in poloxamer 407 gel (Otiprio, Otomomy Inc.) and has gained favor in veterinary medicine. The use of topical therapies is based on the high levels of antibiotic that can be placed into the bulla coupled with the poor drainage of the tympanic bulla. Aqueous solutions of, or
20% poloxamer 407 compounded with, non-toxic antimicrobials can be placed directly into the bulla, typically guided by video otoscopy. Infused antibiotics can remain in contact with the inflamed granulating middle ear mucosa much longer (seven days or longer for poloxamer-based formulations), because the fluid filling the bulla cannot readily escape. Failure may result from inability of the antibiotic to penetrate to sequestered bacteria within folds or pockets of granulation tissue, or failure to penetrate successfully to a diseased ventromedial compartment past the bulla septum. Safety and success rates for this treatment in cats have not yet been reported, though anecdotally 1.5% enrofloxacin / 2% ketoconazole / 0.1% dexamethasone in poloxamer 407 has been used safely and with success in several cases of feline OM in the author’s practice.

Table 3: Topical Therapies Safe for Middle Ear

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Antifungals</th>
<th>Anti-inflammatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroquinolones</td>
<td>Azoles</td>
<td>Glucocorticoids</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Clotrimazole</td>
<td>Aqueous dexamethasone</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>Ketoconazole</td>
<td>Aqueous fluocinolone</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td></td>
<td>Miconazole</td>
</tr>
<tr>
<td>Penicillins</td>
<td>Others</td>
<td></td>
</tr>
<tr>
<td>Penicillin G</td>
<td>Nystatin</td>
<td></td>
</tr>
<tr>
<td>10% Ceftazidime</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11.25%/22.5% Piperacillin-tazobactam</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other

| Triz EDTA            |                  |                  |
| Otoflush (Dermcare Pty Ltd)^ |            |                  |
| 4-13% aluminium subacetate # |                |                  |

* minor hair cell loss on 2/24 chinchillas  
# no significant changes in guinea pigs  
^ no significant changes in dogs

Proliferative Necrotising Otitis. This is a clinically distinctive though rare disease of cats consisting of bilateral, well-demarcated dark brown to black, proliferative, often plaquelike coalescing lesions usually (though not always) covering the concave surface of the pinnae potentially extending into and obstructing the vertical ear canal. The proliferative tissue is friable leading to erosion and ulceration with a thick exudate. Secondary bacterial or yeast otitis externa frequently accompany the lesion. Pruritus and pain are usually minimal, and occur most frequently in cases that have advanced to ulceration and infection.

It is more common in kittens, but cases in adult cats up to 14 years of age have been reported. Lesions typically first appear in kittens aged 2 to 6 months and completely and spontaneously regress by two years. However, spontaneous resolution does not occur in all cases, with some cases reported persisting up to four years.
On histopathology, the lesions are characterized by a markedly hyperplastic epidermis with pronounced hair follicle outer root sheath hyperplasia and neutrophilic luminal folliculitis, follicular keratosis and individually apoptotic-appearing keratinocytes in the outer root sheath of hair follicles. Immunohistochemistry (IHC) has shown a close association between CD3+ T cells and caspase-3 stained keratinocytes, consistent with a keratinocyte apoptosis by epidermal-infiltrating T cells, but the trigger for this is not clear. A viral etiology has been suggested but investigation by PCR and IHC has not supported this.

Topical 0.1% tacrolimus twice daily has been the most successful treatment reported to date, with resolution apparent as soon as 2 to 3 weeks in some cases. Some animals may need treating for months. In these latter cases, it is unknown if resolution was due to the medication or was spontaneous. Topical and systemic glucocorticoids and antibiotics have not been as reliable, with multiple failures reported. However, occasional cases have responded. One case used 2mg/kg intralesional methylprednisolone acetate followed with topical 0.05% clobetasol propionate daily and systemic antibiotics. A second case responded to nine weeks of daily topical 0.1% mometasone furoate, followed by 4 weeks of oral triamcinolone 0.9mg/kg daily to every second day, and a further two weeks of 2mg/kg prednisolone every second day.

**Feline Nasopharyngeal Polyps.** Feline nasopharyngeal polyps (FNPs, also known as respiratory tract polyps, pharyngeal polyps, or middle ear polyps) are nonneoplastic inflammatory lesions that arise from the mucosal lining of the middle ear, Eustachian tube, or pharynx. They are the most common benign pharyngeal and external ear canal masses diagnosed in cats and this was also found in the ASES study above. Nasopharyngeal polyps may also be found in the dog, but are less common than in the cat.

The exact origin and cause of FNPs is unknown though multiple theories have been proposed:

- a congenital etiology (given the increased frequency in young cats) in which these polyps may arise as aberrant growths from the remnants of the branchial arches
- the result of a prolonged inflammatory process. It is unclear whether this inflammation initiates or potentiates the development and growth of the polyps.
- secondary to an infection ascending from the nasopharynx. The typical inflammatory changes seen histopathologically combined with clinical signs in these patients may imply a viral or bacterial contribution. Two studies have recovered feline calicivirus from the polyps of some affected cats. However, a third study found no evidence for feline herpesvirus-1 or calicivirus in 41 polyps using PCR, suggesting that tissue persistence of these viruses is not essential to polyp formation, although immunologic clearance of these viral organisms before polyp diagnosis remains a possibility. Furthermore, it is unclear whether inflammation and infection themselves are primary or secondary to FNP formation.
- secondary to chronic upper respiratory tract inflammation
- secondary to chronic OM. While there is no direct evidence for this in cats, polyps have been experimentally induced in rats with placement of type three pneumococci in the bulla. That study proposed a theory of polyp pathogenesis, based on the following stages: 1) Localized rupture of the epithelial lining, 2) Luminal protrusion of the lamina propria through the epithelial defect, and 3) Re-epithelialization of protruded tissue and formation of a polyp.7
FNPs should be differentiated from inflammatory polyps that can occasionally arise from the external ear canal. These latter lesions tend to be more prednisolone responsive and show a different histopathology typified by stratified epidermis overlying variably ceruminous gland hyperplasia, fibrous tissue and mixed inflammatory cells, compared with the pseudostratified ciliated columnar epithelium overlying variably inflamed fibrovascular connective tissue seen in FNP.

The typical presentation is that of a cat generally younger than 3 years of age, though cats of any age may be affected. No sex predilection has been identified, though various studies have found breed predilections for Maine Coon cats, domestic shorthair cats, Abyssinian cats or no breed predilection. Polyps may be unilateral, or less commonly bilateral, and can extend through the tympanic membrane to the horizontal canal, or can extend through Eustachian tube to nasopharynx.

Clinical signs may be present for weeks to years before diagnosis and signs vary depending on the location of the polyp (the bulk of the polyp may be located in the pharynx, in the middle ear or in the external ear canal) and the location of any associated secondary infection.

- **Lesions in the nasopharynx**: May present with upper respiratory signs including nasal discharge, epistaxis, weight loss, sneezing, stertor, voice change, dyspnea and dysphagia. Less commonly, cyanosis and syncopal episodes may occur.
- **Lesions in the middle ear**: May present with signs consistent with OM or OI including head tilt, loss of balance, nystagmus (vestibular signs may be due to pressure on the round / oval windows increasing pressure on endolymph in semicircular canals), keratoconjunctivitis sicca, facial nerve paralysis, and loss of blink reflexes, deafness and Horner’s syndrome.
- **Lesions in the external ear**: May present with otic discharge, head shaking, otic pruritus, and non-neurological head tilt secondary to pain and fluid. A mass may be visible when the polyp protrudes through the tympanic membrane, but a visible polyp is reported in less than half of cats.

Diagnosis is generally obtained with a combination of signalment, history and clinical signs, visual observation of a mass (otoscopic or oropharyngeal examination – retraction of the soft palate using a spay hook can be useful), skull radiographs, advanced imaging (CT, MRI), and histopathology following removal. The observation of a smooth to fleshy pink mass in the external ear of a young cat alone would be strongly suggestive of the diagnosis.

The treatment of FNPs is primarily surgical. The most common technique is traction and avulsion (TA) of the polyp otoscopically, via a lateral surgical approach or via VBO to also remove the epithelial lining of the tympanic bulla. Traction is preferred to incision because it is more likely to remove the peduncle, and destroy the blood supply, reducing the relapse rate.

TA is the least invasive technique, yet has the highest documented recurrence rate (up to 57%) though better results have been reported using a per-endoscopic transtympanic traction technique, which resulted in polyp recurrence in only 13.5% of the 37 cats treated. Visualization
is critical using this technique to ensure the polyp is grasped as deep as possible and that all tissue is removed. Partial removal is likely to result in regrowth, and relapse of clinical signs. Complications are typically infrequent with these techniques (Horner’s syndrome in 8% of cases in the endoscopic study above, though up to 43% has been reported).

TA following a lateral surgical approach to the horizontal canal showed a recurrence rate of polyp regrowth of 14-35%. Postoperative complications included Horner’s syndrome (11.5%) and facial nerve paralysis (3%).

VBO is the most invasive technique with the highest associated complication risks but with the lowest published recurrence rates (0–8% for most studies). Some authors recommend that if signs indicative of OM are present, ventral bulla osteotomy should be advised. Complications may include Horner’s syndrome, OI, facial nerve paralysis (58%, 11% and 26% respectively in one study) and hypoglossal neuropathy. A high percentage of these complications are self-limiting.

Concurrent OM/OI is a common finding and bacterial culture followed by appropriate antibiotic therapy is recommended. Use of post-surgical corticosteroids is controversial. One study found that postoperative anti-inflammatory doses of prednisolone significantly reduced the rate of recurrence of polyps after traction, although the location of the polyp (pharyngeal versus aural) in patients that received prednisolone was not clearly defined. Topical administration of glucocorticoid solution into the bulla otoscopically post surgically has been recommended. Other authors state that topical or systemic corticosteroid administration is not beneficial and is contraindicated.

The prognosis is excellent if there is complete removal of the polyp and infection can be appropriately addressed. Polyps of nasopharyngeal origin may be less likely to reoccur than ones of middle ear origin.

References
VENTRAL ALOPECIA – IS IT DERMATOLOGICAL, BEHAVIORAL OR MEDICAL?

Danny W. Scott
Department of Clinical Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, USA

For these purposes, the discussion will be limited to cats that are examined for hair loss (hypotrichosis and/or alopecia) confined to the ventrum and with no macroscopic signs of inflammation. Such cats accounted for 9% (123 of 1407) of the feline patients examined by the dermatology service of a university companion animal hospital.²

The most common cause of this cutaneous reaction pattern is cats that are overgrooming, usually because of allergies (atopic dermatitis, food allergy) (Table 1 and Table 2).¹,² In these instances, visible hairs appear broken off, of varying lengths and do not easily epilate. Much less commonly, the hairs are actually falling out, typically due to medical disorders (hyperadrenocorticism, diabetes mellitus, paraneoplastic alopecia) (Table 1 and Table 2).¹,² In these instances, visible hairs usually appear normal (occasionally miniaturized), of similar lengths and area easily epilated.

A thorough dermatological and medical history, physical examination and trichography will usually distinguish between overgrooming versus spontaneous hair loss. Further laboratory evaluations will be based on a prioritized differential diagnosis.

Table 1: Causes of Ventral Alopecia in Cats¹

<table>
<thead>
<tr>
<th>Dermatological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatitis</td>
</tr>
<tr>
<td>Food allergy</td>
</tr>
<tr>
<td>Demodicosis (D. gatoi)</td>
</tr>
<tr>
<td>Otodectic mange</td>
</tr>
<tr>
<td>Cutaneous adverse drug reaction</td>
</tr>
<tr>
<td>Medical</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
</tr>
<tr>
<td>Hyperadrenocorticism</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Paraneoplastic alopecia</td>
</tr>
<tr>
<td>Focal pain (e.g., cystitis)</td>
</tr>
<tr>
<td>Behavioral</td>
</tr>
<tr>
<td>Behavioral (“psychogenic”) overgrooming</td>
</tr>
</tbody>
</table>

¹ Numbers in superscript refer to the specific references.
² Numbers in superscript refer to the specific references.

1, 2
Table 2: Prevalence of Causes of Ventral Alopecia in Cats (123 Cases)²

<table>
<thead>
<tr>
<th>Disorder</th>
<th>% of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatitis</td>
<td>73.0</td>
</tr>
<tr>
<td>Food allergy</td>
<td>16.0</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>5.0</td>
</tr>
<tr>
<td>Behavioral</td>
<td>2.0</td>
</tr>
<tr>
<td>Hyperadrenocorticism</td>
<td>1.5</td>
</tr>
<tr>
<td>Paraneoplastic alopecia</td>
<td>1.5</td>
</tr>
<tr>
<td>Cystitis</td>
<td>1.0</td>
</tr>
</tbody>
</table>

References
DERMATOPHYTOSIS TREATMENT – WHAT WORKS?

Kimberly S. Coyner
Dermatology Clinic for Animals, Lacey, WA USA

Introduction
Treatment of dermatophytosis in small animal patients can be an intimidating prospect in some cases, but this disease is actually not difficult to treat if we know how to accurately diagnose it, what topical and systemic treatments are effective and how to monitor and determine successful treatment.

Overview of Dermatophytosis
There are over 30 species of dermatophytic fungi, but the most common dermatophytes that cause disease in dogs and cats are Microsporum canis, Microsporum gypseum (soil origin), and Trichophyton mentagrophytes (rodent origin).

Transmission and risk factors for infection: Dermatophytes are most commonly transmitted by direct contact between an infected and uninfected animal; fomite transmission can also occur, including grooming equipment, bedding, collars, ectoparasites and exposure to a contaminated environment. Risk factors for dermatophyte infection include warm, humid environments, young age and group housing (shelters, catteries, and canine day care). Immunosuppressive diseases such as hyperadrenocorticism may play a role in some cases. Persian cats and Yorkshire terriers are predisposed to dermatophytosis.

Clinical signs in cats: Focal to multifocal to extensive regional or generalized alopecia with variable crusting, scaling, erythema, follicular cast formation, comedones and hyperpigmentation; pruritus may range from none to severe and in some cases military dermatitis, symmetrical alopecia, and eosinophilic plaques similar to hypersensitivity dermatitis can occur. Lesions are commonly first noted on the face and limbs but can occur in any area. Alopecia, scaling or crusting of one or both outer pinnae can occur, as can chin dermatitis similar to feline acne.

Clinical signs in dogs: Often focal to multifocal areas of alopecia with variable scaling, crusting, papules and hyperpigmentation which are clinically indistinguishable from more common bacterial pyoderma and demodecosis. Facial and muzzle crusting and furunculosis which can mimic immune-mediated diseases. Dermatophyte kerions are localized raised nodular exudative inflammatory lesions most commonly caused by Trichophytton spp. and Microsporum gypseum. Nail involvement is characterized by onychogryphosis on one to multiple toenails.

Diagnosis of Dermatophytosis
The diagnosis of dermatophytosis is not made by any one single diagnostic test but rather by ruling out other causes for the lesions such as a bacterial pyoderma and demodecosis and by appropriate tests to screen for and identify dermatophytes. Diagnostics for dermatophytosis are divided into two major categories: ‘Point of Care’ tests and ‘Confirmatory’ testing, which confirms and identifies dermatophyte species.
Point of Care Diagnostics

Wood’s Lamp Examination: A Wood’s lamp is a tool that can be helpful to look for fluorescing hairs infected by *M. canis* and to collect samples for microscopic examination, dermatophyte culture and/or PCR; dermatophyte species other than *M. canis* do not fluoresce. False positive and false negative results are commonly due to inadequate equipment, lack of magnification, patient compliance, poor technique or lack of training. Many other skin diseases and artifacts can create colored phosphors, including pyoderma, *Demodex*, keratin, soap, topical medications, and carpet fibers. This can lead to errors in Wood’s lamp interpretation. However *M. canis* produces a distinct apple green color which tracks along hairshafts. Use a medical grade plug in the Wood’s lamp (320 to 400 nm) with built-in magnification. Examine in a dark room and hold the lamp close to the skin (2-4 cm). Infected hairs are often obscured by scale and crusts; be sure to gently lift crusts and scales and look under the crusts. Newly infected hairs can be very short.

Microscopic Direct Examination of Hairs and Scale: The best way to collect specimens is via both superficial skin scraping and plucking of hairs from lesions. Use mineral oil as a mounting medium; clearing agents such as KOH can induce artifacts, destroy fluorescence and damage microscope lens. Use a glass coverslip and examine at 4x and 10x magnification looking for abnormal hairs which appear wider and paler than normal hairs. Beware of saprophytic fungal spores such as *Alternaria* spp., which are commonly found on the fur. Dermatophytes do NOT form macroconidia on the skin/fur, only in culture. Dry scrapings/cytology of lesions stained with Diff Quik can sometimes show dermatophyte arthroconidia, hyphae or fragmented hyphae.

Confirmatory Tests

Dermatophyte culture: Dermatophyte test medium (DTM) consists of a nutrient medium plus inhibitors of bacterial and saprophytic growth and phenol red as a pH indicator. Round or rectangular dermatophyte culture plates are superior to the screw top vials and can be ordered from most veterinary distributors. Collect fungal culture specimens by vigorously brushing lesions (both fur and skin) with a soft bristled new toothbrush then gently press bristle tips into the culture media. Do not pluck hairs from the toothbrush for culture as this has been shown to be inferior and lead to more contaminant growth. Dermatophyte colonies may appear as soon as 5 to 7 days after inoculation; *Trichophyton* spp. often grows and sporulates more slowly. Most *M. canis* cultures are positive by day 14. Plates should be inspected daily for growth; dermatophytes are white or tan in color; they are never green, gray, brown, or black. The red color change is caused by a change in the pH of the medium as dermatophytes metabolize protein in the culture medium. The color change usually occurs at the same time or within 24 hours of dermatophyte colony growth. Saprophytic fungi will eventually produce a red media color change after the colony has grown for several days to a week; occasionally a saprophyte can cause media color change concurrent with fungal colony growth. Red color change alone is NEVER diagnostic. Microscopic confirmation is always required to confirm true infection.

Dermatophyte culture cytology: To evaluate suspicious fungal colonies, make a clear (not frosted) acetate tape preparation, touch the tape to the top of the fungal colony then place the tape on a microscopic slide over a drop of lactophenol cotton phenol blue stain or Diff Quik blue thiazine stain and observe under 10-40x magnification for macroconidia:

- *M. canis*: > 6 cells, canoe shaped, thick walls, terminal knob.
- *M. gypseum*: < 6 cells, canoe shaped, thin walls, no knob.
**T. mentagrophytes**: thin walls, cigar shape, few spores; spiral hyphae often present.

Beware of contaminant fungi which can have spores similar to dermatophytes such as *Alternaria* and *Fusarium* species. If the identity of the fungus cannot be confirmed then send the dermatophyte culture to a reference lab for fungal identification.

**Dermatophyte Polymerase Chain Reaction (PCR)**: PCR testing has the advantage of rapid turnaround for results. Active dermatophyte infection is present in the hair follicle and epidermis, so to avoid false negative results it is important to submit an adequate sample including hair roots and epidermal debris (scales and crusts). Hairs can be plucked with forceps and transferred to a sterile container. Alternatively, lesions can be aggressively combed with a sterile toothbrush and accumulated hair/skin debris submitted in a sterile tube for PCR analysis. It is important to remember that PCR testing cannot differentiate fomite carriers from truly infected individuals. Because the PCR test detects both viable and non-viable fungal DNA, false positive test results can be found in animals with a mycological cure determined via fungal culture. A negative PCR test in a treated animal with a clinical cure can be used as an indication of mycological cure. However, if the PCR test is positive then a dermatophyte culture should be performed to determine if the PCR detected viable or non-viable fungal DNA.

**Treatment and Monitoring**
Although dermatophytosis is a self-limiting disease in immunocompetent animals, treatment is recommended to shorten the course of infection because it is an infectious, contagious and zoonotic disease. In refractory generalized cases of dermatophytosis, investigate underlying immunosuppressive disease such as FeLV/FIV infection, hyperadrenocorticism, hypothyroidism or neoplasia. Optimum treatment involves the use of topical antifungal therapy to disinfect the hair coat combined with a systemic antifungal drug to eradicate the infection in the hair follicle.

*Is clipping needed?* Clipping of the entire hair coat is not needed as a general recommendation and may worsen clinical signs and spread the disease. Long haired adult cats and/or Yorkshire terrier dogs may need to be clipped if the infection is not easily eradicated, recurrent, and/or if matted or thick fur make it difficult to thoroughly soak the hair coat and the surface of the skin. Pre-treatment with 1 to 2 lime sulfur rinses or enilconazole before clipping can minimize contamination of the environment during the clipping process and clippers should be discarded after use or carefully cleaned and gas sterilized.

**Topical Antifungal Drug Treatment**
Topical therapy should be used in all cases of localized and generalized dermatophytosis in dogs and cats. It disinfects the hair coat, which is the source of infective material for other animals and people, and minimizes shedding of infective spores in the environment, thus minimizing the potential of false positive fungal cultures and subsequent unnecessarily prolonged treatment.

*Focal lesions*: Focal dermatophyte lesions can be treated with a topical antifungal cream, solution or ointment (clotrimazole, miconazole, terbinafine, or climbazole) once daily until negative culture. Hair around focal lesions can be clipped with round tipped scissors to facilitate medication application.
Generalized lesions: In animals with multifocal or generalized lesions, topical whole-body antifungal therapy should be combined with systemic antifungal therapy. Twice weekly whole-body rinses or antifungal shampoos are recommended for the duration of therapy of infected animals; leave-on dips have more residual activity and are preferred over shampoos. Additionally, topical whole-body rinses or antifungal shampoo treatments help minimize the risk of transmission to other animals in a household and can be used to treat exposed uninfected animals preventatively.

Topical Antifungal Options for Generalized Disease

Lime sulfur: This dip is fungicidal due to the formation of hydrogen sulphide. Most veterinary commercial formulations of concentrated lime sulfur contain 97.8% saturated lime sulfur which is diluted with water, 4-8 ounces dip/gallon of water (15-30ml of dip/L of water). In some countries, it is labelled as 23% calcium polysulfide or 23% sulfur sulfide; this is equivalent to 79.9% lime sulfur solution, which is still diluted 1:16 to make a 5% dilution. The diluted solution is applied all over the body (taking care to avoid the eyes/ rinse eyes with saline if the dip accidentally drips into eyes) using a sponge or low pressure pump garden sprayer and then let dry (do not rinse). The leave on rinses should be applied twice weekly and prepared fresh each time. It is very important to keep kittens and small puppies warm, as they can easily become hypothermic. Potential temporary adverse effects include drying of the footpads and haircoat, loss of hair on the ears, and yellow discoloration of the hair coat of white cats. Licking of the dip can incite nausea and hypersalivation, but does not cause oral ulceration.

In one study, 58 shelter cats were treated with oral itraconazole and twice weekly lime sulfur dips; the mean number of days to cure was 18 and all cats were cured by day 49 after starting treatment. In another study, 85 shelter cats treated with twice weekly lime sulfur and a three-week course of oral terbinafine. Mean time to mycological cure was 22.7 days; when lime sulfur rinses were decreased to once weekly there was an increase in the number of fungal colonies cultured — cats did not cure until twice weekly applications were used.

Enilconazole: This product is available in Canada and Europe as a 10% concentrated solution (Imaverol™, Elanco). It is an imidazole antifungal which inhibits ergosterol synthesis and damages fungal cell membranes. To use, dilute 1 part Imaverol in 50 parts lukewarm water (e.g., dilute 20ml Imaverol fungicidal wash in 1 liter water to make a 0.2% enilconazole solution). Dilute fresh for each use; the shelf life of an opened bottle is 3 months. Sponge the diluted enilconazole all over body and let dry. Place an Elizabethan collar to prevent licking until dry and make sure to keep kittens and small puppies warm. Potential temporary adverse effects include drying of the haircoat and occasional liver enzyme elevation. A shelter study used stringent environmental decontamination and animal location stratification according to lesional and culture status. Cats were treated with oral itraconazole and twice weekly 0.2% enilconazole dips. Twenty-four clinically affected cats and 22 lesion-free carrier cats were cured and culture negative after 30 to 56 days of treatment.

Combination miconazole/chlorhexidine shampoos: If antifungal dips are not available or not an option, then twice weekly 2% miconazole/2% chlorhexidine shampoos can be used; the shampoos require 5-10 minute contact time before rinsing and (unlike dips) do not have residual antifungal activity. The combination of chlorhexidine and miconazole was shown in one small
study to be superior to miconazole shampoo alone; chlorhexidine alone is not effective for treating dermatophytes. 1% ketoconazole/2% chlorhexidine and 0.5% climbazole/3% chlorhexidine shampoos show promise in vitro. Additionally, chlorhexidine/azole mousse applied every other day may be an option for pets with limited lesions, or fragile/fractious pets, which cannot be dipped or bathed.

Systemic Antifungal Drug Treatment Options
Systemic antifungal therapy eradicates the fungal infection within the hair follicle and is used in animals with generalized dermatophytosis in conjunction with topical antifungal therapy and environmental cleaning. Recommended/first line systemic treatment options are either itraconazole or terbinafine.

Itraconazole is an imidazole antifungal medication which inhibits ergosterol synthesis and damages fungal cell membrane. It labelled in cats as a liquid formulation (Itrafungol®, Elanco) dosed at 5 mg/kg administered once daily on an alternating week on/week off treatment schedule for three cycles or until mycological cure; in dogs give itraconazole daily until cure. The human brand name or generic 100mg itraconazole capsules can be used at a dose of 5 mg/kg, but compounded itraconazole has been shown to have poor bio-availability and is not recommended. An acidic environment is required for maximal absorption, so avoid concurrent medications that reduce stomach acidity. Adverse effects of itraconazole are uncommon and can include salivation, mild anorexia, and vomiting; elevations in hepatic enzymes are uncommon and are dose related/more common in animals being treated for deep fungal infections with prolonged higher itraconazole doses. In animal safety studies, kittens as young as 10 days of age were treated with 4 weeks of itraconazole with no adverse effects, but itraconazole should not be used in pregnant animals due to risk of fetal toxicity, and use with caution in patients with decreased cardiac function due to possible negative inotropic effects.

Terbinafine is an allylamine antifungal drug which inhibits fungal sterol biosynthesis and causes fungal wall damage. It has the lowest MIC for Microsporum and Trichophyton species when compared to itraconazole, fluconazole, ketoconazole or griseofulvin. Dosage is 30-40mg/kg/day. After 2-3 weeks of daily dosing in cats, begin week on/week off pulse treatment until cure. Continue daily in dogs until cure. Potential side effects include occasional vomiting, diarrhea and soft stools.

Second Line/Less Recommended Systemic Treatment
Ketoconazole has a good spectrum of activity against dermatophytes but is not a first-choice systemic antifungal drug. This is a drug option for dogs only. The drug is not well tolerated in cats and causes vomiting, diarrhea, anorexia, potential hepatotoxicity and weight loss with prolonged use.

Griseofulvin is an older antifungal drug with efficacy similar to ketoconazole but is less effective and much slower in activity than terbinafine or itraconazole. Griseofulvin damages fungal cell walls and may antagonize chitin synthesis in the fungal cell wall by inhibiting nucleic acid synthesis and cell mitosis. It is a teratogen and should not be used in pregnant animals. Griseofulvin needs to be administered with a fatty meal to enhance absorption. Vomiting and diarrhea are the most common side effects associated with administration. Lethargy and
hepatopathy can also occur, as well as, idiosyncratic bone marrow suppression, especially in cats with retroviral infections. Complete blood counts should be monitored regularly during treatment with griseofulvin.

**Poorly Effective/Not Recommended Systemic Treatment**
Fluconazole has poor efficacy against dermatophytes and it has the highest MIC compared to itraconazole, terbinafine, ketoconazole and griseofulvin for both *Microsporum* spp and *Trichophyton* spp. It is not recommended for treatment of dermatophytosis. Lufenuron has no efficacy in the treatment of dermatophytosis and should not be used.

**Dermatophyte Vaccines**
Multiple inactivated vaccines (*M. canis* or mixed dermatophyte species) are available in different European countries. Studies with the various products have shown differing efficacy. In general, antifungal vaccines do not protect against challenge exposure in small animal patients but may be a useful adjunct therapy to abbreviate disease when used with topical and systemic antifungal medications.

**Recommendations for Monitoring**
Clinical cure will precede mycological cure and the first post treatment examination should occur within 4 weeks. If new lesions are still developing, client compliance with treatment should be reviewed. In animals with *M. canis* infections, examinations should include a careful Wood’s lamp examination. Treatment should be continued until the animal is culture negative or is negative on PCR. In animals with simple infections, one negative dermatophyte culture may be sufficient. In animals with complicated infections, two negative fungal cultures may be needed, especially if the animal is long haired. Topical therapy should be continued pending the fungal culture or PCR results. Animals can be considered mycologically cured if there is a negative PCR test. If PCR is positive, a fungal culture should be performed to determine if the test was detecting viable spores or nonviable fungal DNA.

*Dermatophyte CFU:* When performing dermatophyte cultures in-house, the number of colony forming units on a fungal culture is very important in the interpretation of response to treatment. Simple results such as “positive” or “negative” are not adequate to determine cure.
- < 5 colonies means the cat is likely cured but picking up spores from the environment or other cats — keep treating topically and review environmental decontamination and culture in contact pets. Reculture in 1-2 weeks.
- 5-10 colonies (grey zone). Continue both topical and oral treatment as well as environmental decontamination. Reculture in 1-2 weeks.
- > 10 colonies means active infection. Continue both topical and oral treatment as well as environmental decontamination. Reculture in 2 weeks.

**Duration of Treatment**
Treatment duration will depend upon the overall health of the patient. Simple infections are those that occur in otherwise healthy animals and these will resolve quickly, often within 3-6 weeks. Complicated infections can take several months to clear and occur in animals under physiological stress, concurrent illnesses or those receiving immunosuppressive medications.
Conclusions
Dermatophytosis is not so scary as we thought! When the diagnosis is accurate, combinations of effective topical and systemic antifungal therapies are used, and treatment efficacy appropriately monitored, this is a very treatable and curable disease.

Selected References
Pododermatitis is an uncommon cutaneous reaction pattern in cats.\textsuperscript{1,2} The paw may be affected along with other body sites in many feline dermatoses.\textsuperscript{1,2} This discussion will be limited to conditions wherein only the paw(s) is(are) involved. I am not going to discuss neoplasms, hamartomas and cysts. Disease may be isolated to the clawbed, claw, pawpad, interdigital space, or combinations of these.

The differential diagnosis for pododermatitis in cats is lengthy (Table 1). Asymmetrical disease is often a result of bacterial and fungal infections, parasitic infestations, trauma, and foreign bodies. Symmetrical disease is often associated with immunological diseases (with or without resident bacterial and/or \textit{Malassezia} infections), viral infections, and plasma cell pododermatitis. Nodules, with or without ulceration and draining tracts, are often due to bacterial and fungal infections. Pawpad involvement is most often seen with pemphigus foliaceus, plasma cell pododermatitis, idiopathic eosinophilic granuloma and viral infections.

In addition to a thorough history and physical examination, laboratory evaluations (skin scrapings, trichography, cytology, bacterial and fungal culture, skin biopsies, and special procedures [e.g., PCR]) are essential for achieving an accurate diagnosis and developing a targeted therapeutic plan.

References
2. Miller WH, Griffin CE, Campbell KL. \textit{Muller & Kirk’s Small Animal Dermatology}. 7\textsuperscript{th} ed. Elsevier Mosby, St. Louis, 2013.
NODULAR SKIN DISEASES IN CATS

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Introduction
A nodule is a circumscribed solid elevation of the skin >10mm in diameter. It is most commonly formed due to infiltration of inflammatory or neoplastic cells into the deeper layers of the skin i.e. dermis, subcutaneous tissue and muscle. Less commonly, they can be due to deposition of material such as lipid (xanthomas) and calcium (calcinosis cutis, calcinosis circumscripta).

Causes of Nodular Skin Diseases
Causes of nodular skin diseases in cats can be broadly differentiated into inflammatory (infectious vs non-infectious) or neoplastic. The most common infectious organisms would depend on geographical location and hence exposure.

Infectious Causes of Nodular Skin Diseases

<table>
<thead>
<tr>
<th>Bacterial</th>
<th>Fungal</th>
<th>Algal</th>
<th>Oomycetes</th>
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<tr>
<td>Bacterial folliculitis &amp; furunculosis</td>
<td>Dermatophytes</td>
<td>Prototheca</td>
<td>Pythiosis</td>
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<td>Mycobacteria</td>
<td>Sporothrix</td>
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<td>Lagenidium</td>
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<td>Nocardia</td>
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<td>Actinomyces/Arcanobacterium</td>
<td>Subcutaneous: Eumycetoma</td>
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<td>Actinobacillus</td>
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<td>Phaeohyphomycosis</td>
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<td>Hyalohyphomycosis</td>
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<td>Systemic mycoses:</td>
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<td>Cryptococcus</td>
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<td>Blastomycosis</td>
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<td>Histoplasmosis</td>
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<tr>
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<td>Leishmania</td>
<td>Feline infectious</td>
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<tr>
<td>Insect bites</td>
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<td>peritonitis</td>
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<td>Tick bites</td>
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<td>(coronavirus)</td>
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<td>Cuterebra</td>
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<td>Feline Cowpox</td>
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<td>Dracunculiasis</td>
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<td>Viral papillomas</td>
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Non-Infectious Causes of Nodular Skin Diseases

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<tr>
<th>Foreign body reaction</th>
<th>Hypersensitivity</th>
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<tr>
<td>Keratin</td>
<td>Eosinophilic granuloma</td>
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<tr>
<td>Calcinosis cutis/circumscripta</td>
<td>Mosquito hypersensitivity</td>
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<td>Xanthoma</td>
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<th>Sterile granuloma</th>
<th>Immune mediated</th>
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<td>Granulomatous sebaceous adenitis</td>
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<td>Nodular sterile panniculitis</td>
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<td>Plasma cell pododermatitis</td>
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Neoplastic Causes of Nodular Skin Diseases
Histiocytic diseases, mast cell tumor, cutaneous lymphoma, sarcoid, cyst

Diagnostic Approach

History
Travel history: exposure to infectious organisms based on geography
Systemic signs: systemic involvement (infections and neoplasia) vs cutaneous only

Signalment
Age: older cats for neoplasia; any age for infections
Lifestyle: outdoor roaming cats: infections

Physical examination
Location: localized pressure points/trauma: calcinosis circumscripta; generalised for infections/neoplasia

Diagnostic procedures
The recommended diagnostic tests would depend on the clinical suspicion and availability of tests. These include skin scrapings for parasites and trichogram for infectious and parasitic organisms.

Fine needle aspirates of nodules can provide clues to the underlying cause. The aspirated material can be used for cytological evaluation (examining for infectious and inflammatory cells) and for cultures (but tissue samples preferred). Most causes of nodular skin diseases affecting cats require tissue biopsies for histopathology (including special stains for infectious organisms, and immunohistochemistry), tissue cultures (including special media, aerobic, anaerobic, fungal) and PCR. When collecting tissue samples for cultures, the surface of the nodules should be disinfected, and samples taken aseptically to prevent growth of surface contaminants.
Selected References
Introduction
Feline skin lesions most commonly occur due to diseases specifically or primarily targeting the skin. However, healthy skin is dependent on good general body health, and any cause of sub-optimal health can result in skin and/or hair coat impairment. Systemic diseases may produce skin lesions directly, or secondarily due to metabolic or immunosuppressive effects. Skin lesions linked to systemic diseases are frequently non-specific, although characteristic lesions may herald the onset of some systemic diseases. Early recognition of skin changes as markers of systemic disease aids optimal patient outcomes. Careful screening of the history of cats presenting with skin disease together with complete physical examination often provides the most useful clues to the potential for underlying systemic disease. Awareness of the potential for systemic immunosuppression in development of some infectious skin diseases is also important.

A number of dermatological presentations may be associated with systemic diseases.

1. **Dull Unkempt Hair Coat**
   Generalized or regional hair coat quality changes may reflect systemic disease. Dull hair coats (loss of sheen), with variable degrees of oiliness, hair matting, tufts of un-shed hair, and/or scaling can occur with a range of systemic problems. Important considerations include:
   i. **Nutritional Deficiencies.** Dull scaly hair coats may be associated with imbalanced diets due to poor storage (high temperatures), poor manufacturing (inadequate antioxidants to prevent rancidity, not meeting nutritional label claims), purposely low fats (weight management diets) or imbalanced ingredients (home-made). Deficiencies often involve essential fatty acids, and sometimes protein, vitamins and/or minerals.
   ii. **Reduced Grooming Ability** associated with chronic systemic problems can impact normal hair coat appearance. Obesity, physical pain (arthritis, oral cavity diseases), and lethargy/malaise from chronic diseases (e.g., cardiac, renal, hepatic) are all considerations.
   iii. **Hyperthyroidism.** Skin changes reportedly occur in 30-40% of cats with hyperthyroidism, although these changes may be under-reported. An unkempt, matted, or greasy hair coat is most common, linked to reduced grooming. Regional or extensive alopecia from excessive grooming, or prominent claw growth are also reported. Cats more typically present with systemic signs including weight loss despite polyphagia, behavioral changes (especially hyperactivity), polydipsia and gastrointestinal signs (vomiting, diarrhea, voluminous feces). Heralding skin signs may be initially recognised at routine vaccination, with physical examination revealing unkempt hair coats, poor body condition, palpably enlarged thyroid glands (80-90% of cases) and/or tachycardia (~48% cases).
   iv. **Hypothyroidism.** Although naturally occurring disease is rare, hypothyroidism is increasingly recognised in cats following treatment for hyperthyroidism (surgical or irradiation). The most common signs are lethargy, reduced appetite, weight gain and...
subtle skin changes including dull hair coats with scaling, hair matting and excessive shedding. Regional alopecia of the pinnae, pressure points, and caudal back is reported.

**Relevant Diagnostics.** For the cat presenting with a dull unkempt hair coat, initial assessment of history (age, lifestyle, general health, diet) along with complete physical examination are the important first steps. Infectious cutaneous diseases are important considerations in higher risk scenarios (younger animals, multiple pets, poor conditions). External parasites including lice and fur mites (*Cheyletiella*), and some cases of dermatophytosis present as an unkempt coat. Cats living in poorer conditions may have concurrent nutritional deficiencies. Dietary causes are important considerations in all cats with this presentation; those on both homemade diets and commercial foods. If cutaneous and nutritional causes are not readily apparent, further evaluation for signs of systemic disease, including thyroid status, is indicated.

**Treatment Considerations.** If underlying infectious or systemic diseases are not evident, and dietary imbalance is suspected, provision of a higher quality balanced diet and/or additional fatty acid supplementation may be indicated. The omega-6 fatty acid, linoleic acid, is an important component of the stratum corneum for skin barrier function and supplementation may benefit dry scaly skin and hair coats. Evening primrose oil and cold-pressed sunflower or safflower oil are rich sources of linoleic acid. Although there are no clear guidelines for dosage, up to ~400mg/kg has been reportedly used with safety in cats. Commencing supplementation with 100mg/kg evening primrose oil once daily (e.g. 500mg per cat per day), or ~ 2mls/kg cold-pressed sunflower or safflower oil may be effective, with dosage potentially increased if clinical improvement is not apparent within 8 weeks. Increased caloric/fat intake from fatty acid supplementation may exacerbate obesity, and pancreatic, hepatic or gastrointestinal diseases, so careful systemic assessment is prudent before considering fatty acid supplementation.

### 2. Alopecia, Erythema, Scaling, Focal Crusting
Prominent alopecia, erythema, scaling and/or focal crusting are amongst the most common skin presentations in cats, potentially caused by a wide range of diseases. Hypersensitivities are very common considerations, as are infections (bacteria, fungal or parasitic) and, more rarely, autoimmune skin diseases (e.g., pemphigus foliaceus). Systemic diseases may occur with:

i. **Secondary Bacterial Pyoderma (SBP) and Malassezia Dermatitis (MD).** Both infections are now well-recognised in cats, most commonly associated with underlying hypersensitivities, and also with immunosuppression from systemic diseases (including FIV, thymoma and neoplasia). SBP is most common and can present with a wide range of skin lesions, including alopecia, erythema, scaling, papules, crusted papules (miliary dermatitis), erosions, ulceration and crusting. Distribution is usually multifocal, with the face, neck, ventral trunk, and limbs commonly affected areas. **MD** can appear as localized, multifocal or occasionally generalized areas of alopecia, erythema, greasy adherent brown scaling and red-brown skin discolouration. The face, chin, pinnae, ventral neck, ventral trunk, interdigital areas and claw folds are more commonly affected sites. With both infections, pruritus may occur from the infection, independently to the underlying disease. Initial antimicrobial therapies are important to resolve established infections and management of the underlying problem essential for on-going control.
ii. **Demodicosis.** Demodicosis due to the follicular mite *D. cati* is rare in cats; however, it is a hallmark for immunosuppression from underlying systemic disease or drug therapies, including diabetes mellitus, FIV, FeLV, SLE, *Mycoplasma haemofelis* infection, hyperadrenocorticism, and systemic and topical inhalant (fluticasone) steroid therapy. It typically presents with localized well-demarcated alopecia, but may be generalized, with absent or mild pruritus. It is usually readily responsive to miticidal therapy, and relatively inconsequential in many cats, with the underlying disease raising more concern. In contrast, *D. gatoi* is a more recently emerging atypical demodex mite that causes contagious pruritic dermatitis, primarily in healthy cats.

iii. **Exfoliative Dermatosis with Thymoma.** Generalised scaling and patchy alopecia, with or without erythema may occur with feline thymoma. Scaling is typically prominent, often in large white flakes. Pruritus is often absent but may be mild with concurrent *Malassezia* dermatitis. Skin changes may precede systemic signs (lethargy, anorexia and weight loss). An interface dermatitis is present histologically, however similar changes occur without thymoma. Skin lesions resolve with successful surgical excision of tumors.

iv. **Paraneoplastic Alopecia.** This unique feline dermatosis is associated most commonly with pancreatic carcinoma, and also with hepatic neoplasia (hepatocellular or bile carcinoma, hepatosplenic plasma cell tumour), or intestinal carcinoma (one cat). Cats are typically over 10 years of age, and present with prominent alopecia and characteristic smooth shiny skin, that often starts on the ventrum and rapidly progresses over a few weeks. Less readily groomed alopecic regions may have adherent brown scale. The dorsum is often spared, but hair may be dull and thinning. The pathogenesis of the skin changes is unknown. The majority of cats have metastatic disease and many are euthanased or die within 8 weeks of first signs. Characteristic follicular atrophy is often present histologically; however, evaluation for systemic disease is often more prudent.

v. **Other Paraneoplastic Dermatoses** are described in people and occur sporadically in cats. Alopecia in various forms is a common change, with or without other skin lesions or pruritus. Internal neoplasia is a consideration for unexplained or atypical alopecia and/or dermatitis, especially in an older or systemically unwell cat.

vi. **Leishmaniosis** occurs rarely in cats in endemic regions of the world, although cats may have an important epidemiological role. Skin changes include papules, nodules, ulceration and crusting, or subtler erythema, alopecia, and scaling, with the head frequently affected. Diagnosis and treatment of leishmaniosis is often complex, although successful management of feline cases is reported.

vii. **Cutaneous Drug Reactions** occur sporadically in cats, with implicated drugs including antibiotics (B-lactams, sulphonamides), antifungals (griseofulvin), topical skin/ear medications and vaccines. Reported lesions include urticaria/angioedema, erythema/scaling, macules/papules, nodules, skin atrophy, and self-trauma lesions from pruritus. Systemic signs including malaise, pyrexia and anorexia may co-exist. Recent drug history is particularly important for atypical skin presentations. Firm diagnosis of a drug reaction requires withdrawal and provocation testing, which is problematic with severe reactions.

viii. **Systemic lupus erythematosus** is very rare in cats, with subtle skin changes of scaling, alopecia, erosions, and crusting reported. As with SLE in other species, cats may present with malaise, pyrexia, reduced appetite and variable signs of systemic disease from associated renal, neuromuscular, haematopoietic and/or ocular disease. Histopathology
may provide supportive evidence of interface dermatitis and diagnosis is reliant on sufficient consistent evidence of multi-organ disease.

ix. *Feline Leukaemia Virus (FeLV)-associated Giant Cell Dermatitis* is a very rare scaling, alopecic and crusting dermatitis, with or without pruritus, reported in FeLV positive cats. The head (pinnae, pre-aurocular, peri-oral) is frequently affected, with variable involvement of feet, footpads and other mucocutaneous areas. Characteristic ballooning of epidermal and follicular epithelial cells (giant cells) is present on histopathology.

x. *Cutaneous horns* are rare conical or cylindrical keratin collections, most frequent on footpads, or sometimes involving the nasal planum or eyelids. Multiple horns are reported with FeLV infection, and single or multiple localised horns with papillomavirus, actinic keratosis, squamous cell carcinoma (SCC) *in-situ*, SCC or keratinizing acanthoma. Screening for FeLV status is warranted.

xi. *Hepatocutaneous Syndrome (Necrolytic Migratory Erythema; Metabolic Epidermal Necrosis)*. A cutaneous presentation of liver or pancreatic disease occurs sporadically in dogs, and one case is reported in a cat with hepatic carcinoma. The cat had painful crusting and excessive scaling of footpads and concurrent signs of systemic illness (weakness, anorexia, vomiting).

**Relevant Diagnostics.** Skin cytology is important to screen for infectious agents, with adhesive tape impressions most useful for superficial bacterial, yeast infections, and also identifying dermatophyte spores. Deep skin scrapings will identify *D. cati*. Screening for underlying immune compromise may be important with infectious dermatoses, particularly in older cats or with unexplained recurrences. Skin biopsies may be indicated if autoimmune diseases are more likely (e.g., pemphigus foliaceus), or for any unusual presentations; however, initial systemic evaluation (blood and urine testing +/- body imaging) is often prudent prior to considering skin biopsies in this scenario and may provide more specific information.

3. **Pruritus**

Feline skin disease with prominent pruritus is common, although most typically associated with hypersensitivities or some infectious dermatoses (e.g., bacterial pyoderma, *Malassezia* dermatitis, *D. gatoi*, herpes facial dermatitis), and rarely with systemic diseases. Although pruritus may be present with secondary bacterial and/or yeast infections, when associated with underlying systemic diseases any pruritus is rarely severe, and more typically manifests with licking, limb shaking, rubbing, or excessive grooming rather than severe scratching and self-trauma.

**Relevant Diagnostics:** An efficient diagnostic evaluation for the pruritic cat encompasses initial key historical screening for allergic or parasitic disease clues, often followed by basic skin sampling to screen for primary or secondary infections. A more exhaustive diagnostic evaluation may be indicated if initial assessment provides conflicting data or with atypical presentations.

4. **Erosive Ulcerative Dermatitis**

Prominent skin erosions and ulceration not explained by self-trauma is a relatively rare presentation in the cat. Firm adherent crusts may overly these epidermal defects. Histopathology is diagnostic for many causes and there are often systemic disease considerations. Diseases
already discussed including bacterial pyoderma, SLE and leishmaniosis may occasionally present as erosive and ulcerative dermatoses. Other considerations include:

i. **Skin Fragility.** This syndrome has multiple potential causes that result in skin tearing associated with minor skin trauma, producing large regions of skin avulsion/ulceration.
   a. **Hypercortisolism.** Spontaneous hyperadrenocorticism is rare and typically occurs with pituitary (most commonly) or adrenal neoplasia in older cats. As cats are clinically tolerant of high cortisol, polyuria, polyphagia and weight loss are often absent unless there is concurrent diabetes mellitus. Iatrogenic hypercortisolaemia is more common, associated with injectable, oral or topical glucocorticoids. Thin, fragile, and easily bruised skin, with variable alopecia is reported in approximately 50% of spontaneous and most iatrogenic cases. Characteristic curling and alopecia of the ear pinnae may also occur, particularly in iatrogenic disease.
   b. **Other causes.** Similar skin fragility is reported rarely in multicentric follicular lymphoma, disseminated histoplasmosis and hepatic lipidosis.
   c. **Cutaneous asthenia** is reported in Burmese cats and sporadically in other breeds. In contrast to other forms, it presents from a young age.

ii. **SCC In-Situ with Papillomavirus Infection in the Devon Rex.** This aggressive form of SCC in-situ begins with single to multiple erosive and crusting to nodular lesions from a young age. Lesions develop progressively and may result in metastasizing SCC.

iii. **Paraneoplastic pemphigus** is reported in one cat with a severe erosive and ulcerative dermatosis that began 3.5 weeks after surgical removal of thymoma. Extensive lesions were present on the ventral abdomen, inner thighs and ear pinnae. Myasthenia gravis preceded the skin signs and both changes resolved after several months; presumed to be paraneoplastic diseases due to autoantibodies released prior to excision of the thymoma.

iv. **Erythema multiforme complex (EM) and Toxic epidermal necrosis (TEN)** are considered similar but separate diseases. EM is characterized by multifocal to confluent keratinocyte death (apoptosis), resulting in multifocal to confluent skin and mucosal lesions that vary in severity from mild erythema to full-thickness ulceration. Classical “target” lesions, (erythematous macules that spread peripherally and clear centrally) may be present. Concurrent malaise and pyrexia are common. Sub-classification as EM minor or EM major is based on severity and distribution of lesions and presence or absence of signs of systemic illness. Human EM is most often associated with herpes viral infections, and less often drug reactions. Most cases of EM reported in cats have been drug associated. TEN is also characterized by keratinocyte apoptosis but affects full thickness epidermis/epithelium of skin/mucosae, resulting in life threatening widespread areas of skin and mucosal necrosis. TEN is most often caused by drug reactions. Diagnosis of EM and TEN is confirmed by histopathology. Diagnosis should prompt a thorough investigation for potential drug triggers and/or underlying infectious diseases. Removal of disease triggers may result in disease resolution and improves prognosis.

**Relevant Diagnostics:** A complete history including recent general health and drug therapies, and a full physical examination screening for internal organ disease are initially important. Skin surface cytology from ulcerated lesions is important to screen for secondary microbial infections. Skin biopsies are often indicated, with prior systemic diagnostics (blood, urine testing +/- body imaging). Collecting multiple skin biopsies from a range of lesions, taking care to include intact skin at the borders of any ulcerated areas, will maximize their diagnostic value.
5. **Nodules and/or Nodular Swelling**

Many nodular skin diseases appear similar, despite contrasting etiologies of infectious, sterile inflammatory or neoplastic causes. Optimal treatments, likelihood of systemic involvement and prognoses vary widely, and thus prompt diagnosis is important.

i. **Infectious Nodules.** Some infectious causes have potential for systemic dissemination:

a. **Mycobacteria.**

i. **Common Saprophytic Mycobacteria** (e.g., *Mycobacterium fortuitum*, *M. chelonae*, *M. smegmatis*) cause the most common feline mycobacterial infections, which present as slowly progressive poorly demarcated irregularly nodular regions with punctate draining tracts, most typically in the caudal abdominal and inguinal areas. These mycobacteria are widely distributed in the environment and grow readily in the laboratory. They typically cause localised cutaneous disease in immune-competent hosts, but occasionally cause disseminated infections in immune-compromised hosts.

ii. Lepromatous mycobacteria (e.g., *M. lepraemurium*, *M. species Tarwin*, *M. visible*) produce rare and more variable infections, including both localised skin nodules in healthy cats and widespread but primarily skin-restricted infections in immunocompromised cats. Infection seems associated with rodent or insect bites, but the reservoir of these mycobacteria is currently unknown.

iii. **Other Mycobacteria.** Tuberculous mycobacteria (*M. bovis*; *M. tuberculosis*, *M. microti*) are obligate animal pathogens with high zoonotic potential that are found in restricted geographical regions. They typically infect immuno-compromised cats and produce discrete skin nodules with concurrent body dissemination. Some environmental species with more selective pathogenicity, (e.g., *M. avium complex* primarily infects familial young Abyssinian and Somali cats; *M. genavense* primarily affects old cats with long-standing FIV) also produced discrete intact or ulcerated skin nodules commonly with concurrent systemic infection (respiratory, GIT) and lymphadenopathy.

b. **Nocardia** species are ubiquitous environmental saprophytic bacteria that cause rare infections in immunocompromised cats via inhalation or wound implantation. Progressive irregular nodules and punctate draining sinuses are typical, often with respiratory infection, or widespread dissemination. The extremities, ventral abdomen and inguinal areas are often affected, and lymphadenopathy is common.

c. **Environmental fungi** may also rarely cause discrete skin nodules, most commonly on the extremities (face, pinnae, feet) via penetrating skin wounds. Although many infections remain localised, some geographically restricted fungal species (cryptococcus, sporothrix, blastomycetes, histoplasma, coccidiomyces) can cause serious disseminated infections, often involving the lungs, eyes, and CNS.

d. **Protozoa.** Toxoplasmosis is rare in cats, usually associated with immunocompromise and frequently presents with systemic signs (malaise, fever) +/- respiratory, gastrointestinal, neurological or ocular signs. Skin nodules occur rarely, but are typically multiple, and may ulcerate. Leishmaniasis may occasionally present with nodules and most infections are disseminated.
ii. **Sterile Inflammatory Nodules.**
   
a. **Sterile Panniculitis** in cats may be associated with pancreatic neoplasia, dietary imbalance (vitamin E deficiency) or be idiopathic. All forms present with single or multiple irregularly nodular regions, with or without draining tracts, most frequently on the ventral abdominal or ventrolateral thorax; these lesions are clinically indistinguishable from mycobacterial or nocardial panniculitis.

b. **Xanthomas** occur rarely in cats as multiple pale yellow to pink plaques, or occasionally intact or ulcerated nodules. They are associated with abnormalities in lipid metabolism (hereditary hyperlipidemia, diabetes mellitus). Lesions are most frequent on the head and distal extremities and may be pruritic. Diffuse yellow irregularly nodular lesions are reported in one normolipaemic cat. Histopathology reveals characteristic xanthomatous macrophages with free dermal lipid deposits.

iii. **Neoplastic Nodules.** Although skin neoplasia in cats is more frequently malignant than in dogs, many forms are locally aggressive with a low risk of metastasis. Aggressive forms with a higher risk of metastasis include some mast cell tumors, malignant melanoma and histiocytic sarcomas.

**Relevant Diagnostics:** Any cat presenting with skin nodules or nodular swelling, particularly with multiple lesions, has potential for systemic involvement. Full physical examination including ocular and oropharyngeal examinations, is important. Initial cytology via fine needle aspiration is always indicated and will sometimes confirm a diagnosis or provide diagnostic and prognostic guidance. Multiple samples are ideally obtained from a range of lesions, sampling more peripherally in large lesions to avoid central areas of necrosis, and from any intact areas containing exudate. If cells are not obtained via aspiration, repeated needle fenestration (repositioning the needle tip multiple times within a mass) might provide higher yield. Granulomatous or pyogranulomatous inflammation is common with most of the infectious differentials but may also be seen with sterile inflammation and complicate some neoplastic lesions. Some infectious organisms may be sparse and/or require special stains to visualise (e.g., mycobacteria, nocardia, cryptococcus). Aspirated samples may also be useful for microbial culture. NB Sporotrichosis in cats has serious zoonotic potential from contact of skin lesions and exudate, so appropriate care is important for suspect cases (e.g. any cat with ulcerative nodular lesions, particularly in endemic areas).

Many nodular presentations will require biopsy and histopathology for firm diagnosis, and some infectious causes will require culture or molecular testing to confirm and/or identify infectious agents accurately and to a species level. However, culture is not recommended for some species (sporothrix, blastomycosis, coccidiomycosis and histoplasma) due to aerosol zoonotic risks. Sterile biopsy samples (or portions of samples) can be held frozen (for PCR testing, specialist culture), and refrigerated (for microbial culture), pending initial results from routine histopathology.

**Conclusions**
In older or clearly debilitated cats with skin disease, recognition of potential for underlying systemic disease is often straightforward. Similarly, cats with severe ulcerative or multifocal nodular skin lesions, or with concurrent signs of systemic illness, will more readily prompt systemic evaluation. More challenging is the cat with alopecia, scaling, erythema and/or mildly crusted skin disease, with or without pruritus, where hypersensitivities and infectious dermatoses
are the more common considerations, but systemic diseases occasionally underlie the skin changes. Knowing when screening laboratory testing, body imaging or other systemic diagnostics are indicated is not always readily clear. Occasionally characteristic lesional clues are present. More frequently, accurate suspicion is reliant on searching for suggestive historical and/or physical examination findings, and/or knowledge of greater potential for systemic disease associations with some presentations.

Selected References
FELINE CUSHING’S DISEASE
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Introduction
Feline Cushing’s disease is a rare disorder that causes hypercortisolism with associated physiologic and physical changes in the affected cat. In 1932, an American neurosurgeon Dr. Harvey Cushing described in human beings a clinical syndrome associated with a corticotroph pituitary tumor that was similar to what was described in patients with an adrenocortical tumor.1 The first feline case of hypercortisolism due to an adrenal tumor was reported in 1978.2 Similar clinical signs, including dermatologic changes are seen in cats with hypercortisolism regardless if the hormonal disturbance results from pituitary dependent naturally occurring hyperadrenocorticism, cortisol secreting adrenal tumors or iatrogenic hyperglucocorticoidism. Many of the physiologic effects of hypercortisolism in cats are similar to those in other species but the cat does demonstrate unique clinical signs, and diagnostic testing and treatment differences.

Pathophysiology of Feline Hyperadrenocorticism
Hyperadrenocorticism (HAC) could include the overproduction of any adrenal cortical hormone; it is most often used to refer to the overproduction of cortisol by cells in the zona fasciculata. However, cats can also have adrenal cortical tumors that secrete sex steroid hormones or intermediates that have glucocorticoid activity. Progesterone is a glucocorticoid agonist and it competes with cortisol for binding so if an adrenal tumor makes high amounts of progesterone, that can cause increased amounts of unbound cortisol.

The majority of cats with HAC do in fact have true Cushing’s syndrome and have a pituitary tumor that is autonomously secreting ACTH, ultimately resulting in hypercortisolemia and bilateral adrenocortical hyperplasia.3,4 In approximately 20% of cats with spontaneous HAC, the hypercortisolemia is the result of a functional adrenal tumor that autonomously secretes cortisol. Both pituitary and adrenal dependent HAC are most often due to adenomas, while a minority of cats have carcinomas with adrenal gland carcinomas being more common than pituitary carcinomas.5 Adrenal adenomas or carcinomas cause hypercortisolemia, inhibition of pituitary ACTH production and atrophy of non-neoplastic adrenocortical tissue.3

In some cats, other hormones may also be increased. Pituitary tumors may also secrete growth hormone or alpha melanocyte stimulating hormone,6,7 and some adrenal tumors as stated above can produce progesterone and other sex steroid hormones as well as cortisol.8,9

Iatrogenic Hyperglucocorticoidism
It is important to exclude any possibility of exposure to exogenous glucocorticoids before the diagnosis of feline Cushing’s syndrome is considered. Corticosteroids are prescribed to cats for treating a variety of medical problems and their use in the management of skin disease is common. Although cats are more resistant to the effects of exogenous glucocorticoids than dogs or people, the clinical signs in cats with chronic iatrogenic glucocorticoid administration when
they occur can be similar to those seen with naturally occurring HAC and it is important for the clinician to be aware of the changes seen in cats with hypercortisolism from any cause.

Clinical Presentation
Feline HAC has no breed or sex predilections and typically affects middle aged to older cats, with an average age at diagnosis of between 10 to 13 years.¹⁻³,¹⁰ Cats most often present as unregulated diabetics or having dermatologic disease.¹⁰ Concurrent diseases in cat with HAC in addition to unregulated diabetes mellitus (DM) include secondary infections of the urinary tract, respiratory tract, subcutaneous abscesses, paronychia and bacterial cholangiohepatitis.³,⁵ Other concurrent diseases such as pancreatitis, chronic kidney disease (CKD), hypertrophic or restrictive cardiomyopathy and hyperthyroidism reflect diseases seen more commonly in older cats.⁵,¹¹ In all species, including the cat, spontaneous HAC progresses slowly over months or years before clinical signs advance sufficiently that a diagnosis is made. Polyuria and polydipsia is a common clinical sign but it may be a consequence of concurrent DM or CKD. It is not known if hypercortisolemia in cats can suppress antidiuretic hormone as occurs in dogs.³ Non-diabetic cats with hypercortisolism are reportedly polyphagic.¹¹ When concurrent DM is present, most cats will have weight loss. Muscle atrophy that occurs with hypercortisolemia is due to protein catabolism and it can contribute to weight loss and weakness. The classically described “pot belly” that results from intra-abdominal fat distribution, hepatomegaly and loss of abdominal muscle tone is recognized in cats with hypercortisolism. Cats with pituitary dependent HAC can develop neurologic signs if the pituitary tumor grows large enough.

Dermatologic Clinical Signs
Chronic hypercortisolemia can cause profound changes to the skin with suppressed proliferation of keratinocytes and fibroblasts, resultant epidermal and dermal atrophy, decreased collagen production and diminished elasticity. Follicles arrest in a haired telogen phase and clipped hair coats will be slow to regrow. These physiologic changes to the skin result in alopecia, scaling, thin skin with visible superficial vasculature, poor wound healing, increased susceptibility to bruising, and ventral comedones.³,⁵,¹¹,¹² Acquired skin fragility in cats is associated with HAC, iatrogenic hypercortisolemia 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 tear with simple manipulations, often during restraint or handling. Folded pinnal tips or curling of the pinnal margins can occur in cats with iatrogenic hypercortisolemia, most often in association with the administration of repositol glucocorticoids.³,¹² Hypercortisolism in the cat does not cause calcinosis cutis.

Clinicopathologic Abnormalities
Routine complete blood cell count and serum biochemistry panel in cats with hypercortisolism are often non-specific and can be completely normal.³,¹⁰ Hematological findings are not consistent but a stress leukogram with neutrophilia, lymphopenia and eosinopenia may be evident and some cats may have a mild anemia present.³,⁴ Eighty percent of affected cats have hyperglycemia due to overt diabetes mellitus, transient diabetes or stress hyperglycemia.³ Elevated liver enzymes can occur but since the cat does not have a steroid induced isoenzyme of alkaline phosphatase (ALP) any increases in this enzyme is likely associated with concurrent
diabetes mellitus, pancreatitis or some primary hepatic disease. Urine specific gravity is typically well above 1.020, even if the cat has polyuria. The majority of cats with hypercortisolism have mild proteinuria.\textsuperscript{11}

**Endocrine Tests for HAC**

*Urine Cortisol: Creatinine Ratio (UCCR).* The UCCR is a good screening test for HAC in the cat. However, when interpreting results it is important to remember that the cat has higher UCCR values than dogs and their own reference range.\textsuperscript{3,13} Cortisol can increase with stress and other illnesses so two morning urine samples should be collected at home, which may present challenges for some owners. This is easiest if owners use a nonabsorbent cat litter. Similarly to its use in dogs, the UCCR has good to excellent sensitivity and a normal value rules out the possibility of HAC.\textsuperscript{3} As the test has a low specificity a positive result needs to correlate to history and clinical signs and then to confirm the diagnosis further diagnostic testing. The UCCR can be combined with an oral high dose dexamethasone suppression test (HDDST) to differentiate between adrenal versus pituitary dependent hyperadrenocorticism.\textsuperscript{13} This combined test is done as the HDDST alone does not reliably differentiate between the two forms of HAC in the cat.\textsuperscript{3,4} If the last UCCR measurement after administration of three oral doses of dexamethasone is <50\% of the mean of the baseline UCCR values than the result is consistent with pituitary dependent HAC (PDH).\textsuperscript{13} If the initial two UCCR values are within reference range than HAC is ruled out, so this combined test can be both a screening and a discriminatory test.\textsuperscript{3,13}

*Low Dose Dexamethasone Suppression Test (LDDST).* Dexamethasone suppression tests are used to evaluate the appropriateness of the physiologic feedback of the pituitary gland when an exogenous dose of a glucocorticoid is administered to the animal. The LDDST is the preferred screening test for evaluating cats with suspected HAC as it has high sensitivity and moderate specificity.\textsuperscript{3} Healthy cats require a higher dose of dexamethasone to achieve consistent suppression than what is needed in dogs. Consequently, the dose for a LDDST in cats is tenfold higher at 0.1 mg/kg.\textsuperscript{7} Lack of suppression of cortisol at either 4 hours and/or 8 hours confirms inappropriate functioning of the pituitary adrenal axis and supports the diagnosis of HAC.\textsuperscript{7} In about half of cats diagnosed with HAC using a LDDST, the values obtained can be used to discriminate between PDH and adrenal dependent HAC (ADH). If the 4-hour cortisol value is \( \leq \) 40 nmol/L (1.4 μg/dL) or the 4-hour or 8-hour cortisol value is > 40 nmol/L (1.4 μg/dL) but is < 50\% of the baseline 0 hour value then PDH is diagnosed.\textsuperscript{5}

*ACTH Stimulation Test.* This test has poor sensitivity as a screening test for spontaneous HAC in the cat. It has only moderate specificity and cats with other non-adrenal illness can have post-ACTH stim values outside the reference range. The test is useful for confirming iatrogenic hyperglucocorticoidism, which causes adrenocortical atrophy and failure to respond to ACTH stimulation. It is also the test used to monitor therapy when trilostane is utilized for medical management.\textsuperscript{3,10} If ACTH is administered IM versus IV, peak cortisol concentrations are lower and occur earlier than 60 minutes.

*High Dose Dexamethasone Suppression Test (HDDST).* For this test, 1mg/kg is given IV and serum cortisol values are measured pre-administration and at 4- and 8-hour post-administration.
Only 40 to 50% of PDH cats will show > 50% suppression of baseline cortisol at 4 or 8 hours; it is no longer recommended as a discriminatory test.\textsuperscript{3,4}

**Endogenous ACTH and ACTH Precursors.** Measuring endogenous ACTH can be used as a discriminatory test. Cats with PDH are expected to have normal or increased endogenous ACTH plasma levels, while cats with ADH will have decreased levels. Some normal cats will have ACTH levels below reference range so this is not used as a screening test for HAC. Samples need specific handling; placed in EDTA tubes and kept on ice and spun in a cold centrifuge to separate the plasma and shipped to the lab on dry ice. Improperly handled samples cause falsely low measurements which would incorrectly support diagnosis of ADH.\textsuperscript{4} Endogenous ACTH precursors (pro-opiomelanocortin and pro-ACTH) were reported to be higher in cats with HAC than cats with acromegaly and DM or cats with only DM alone.\textsuperscript{15} However, this is not a commercially available test.

**Diagnostic Imaging**

Diagnostic imaging can provide valuable information to confirm the diagnosis of naturally occurring HAC and to differentiate between pituitary and adrenal dependent HAC. It can also aid in making treatment decisions.

**Abdominal Ultrasonography.** Adrenal glands can be evaluated by abdominal ultrasound. However, it is important to remember that their size can also be influenced by non-adrenal disease, such as hyperthyroidism, which can increase adrenal glands by as much as 20%.\textsuperscript{14} The experience of the ultrasonographer will also influence the sensitivity of this imaging technique as a discriminatory test. One study reported a 93% sensitivity for abdominal ultrasonography to discriminate between ADH versus PDH in cats.\textsuperscript{11} Cats with PDH have bilateral symmetric enlargement of the adrenal glands. Cats with ADH have a visible adrenal mass with typically atrophy of the other adrenal gland. In cats with adrenal tumors, the presence of invasion into nearby vascular structures or evidence of metastatic spread should also be assessed. Occasionally cats can have bilateral adrenal tumors with different functional cell types. The abdominal ultrasound can also evaluate for evidence of pathology in other organs and provide more information about any concurrent diseases.

**Computed Tomography (CT) or Magnetic Resonance Imaging (MRI).** Performing advanced imaging of the pituitary gland is necessary to determine the size of the corticotroph tumor. This is an important diagnostic test to consider, as macroadenomas are present in approximately 50% of cats with PDH.\textsuperscript{5} Confirming the presence of a macroadenoma provides relevant information to guide therapeutic choices since medical treatment does not slow the progression of a macroadenoma and any associated neurologic signs. Advanced imaging of the abdomen can provide more information about adrenal tumor size and degree of tumor invasion particularly nearby vascular invasion that is helpful in surgical staging. It is reasonable to consider performing both imaging studies under one general anesthesia to determine best therapeutic options.

**Medical Treatment of Hyperadrenocorticism**

**Trilostane.** Trilostane is a synthetic steroid analogue that competitively inhibits 3-β-hydroxysteroid dehydrogenase, which is the enzyme needed to convert pregnenolone and 17-α-hydroxyprogrenolone to progesterone and 17–α-hydroxyprogesterone, precursors in cortisol
synthesis. The pharmacokinetics of this drug in cats is not known and recommendations about using it in cats is extrapolated from use in dogs. The drug is given at 1 to 2 mg/kg orally twice a day. Monitoring response to therapy includes assessment of the cat’s activity, appetite, water intake, urination habits and results of an ACTH stimulation test performed 2 to 4 hours after feeding and administration of trilostane. Cats with dermatologic lesions can be monitored for improvement but care should be taken when restraining HAC cats. The extent of adrenal suppression is assessed by the ACTH test and the dose of trilostane is then adjusted. The ACTH stimulation test can be combined with a UCCR test to determine if dose or administration frequency need to be adjusted. Side effects of trilostane include vomiting, diarrhea and hyporexia and may be due to the drug or result of inducing hypocortisolemia. The majority of cats attain some favorable clinical response with trilostane although complete resolution of all clinical signs is rare.

*Lysodren (Mitotane).* Mitotane is an adrenocortolytic drug that is cytotoxic to the zona fasciculata and reticularis layers of the adrenal cortex. It is less effective than trilostane and is not recommended as a first line therapy as it does not often control clinical signs. Side effects of the drug include vomiting and hyporexia these can also be signs seen from the drug itself or if hypoadrenocorticism develops.

*Other Medical Therapies.* The use of other steroid hormone synthesis inhibitors such as metyrapone, ketoconazole and aminoglutethimide have been reported in small numbers of cats with HAC with limited efficacy or development of adverse effects; they are not currently recommended therapies for feline HAC.

**Surgical Treatment of Hyperadrenocorticism**

*Adrenalectomy.* Unilateral adrenalectomy for ADH or bilateral adrenalectomy for PDH are associated with high risk of post-operative complications. Mortality often occurs in the first post-operative week. Bilateral adrenalectomy will require lifelong hormone replacement therapy with both glucocorticoids and mineralocorticoids and it will not alter progression of a macroadenoma. One-year survival rates after adrenalectomy have been obtained in 50 to 70% of reported cases.

*Hypophysectomy.* In theory, transsphenoidal hypophysectomy for PDH can be curative. The procedure has been described in 9 cats with PDH and only 3 cats survived for more than 15 months. Cats will require post-operative hormone replacement therapy with cortisone acetate, levothyroxine and desmopressin. This is not a widely available surgical option.

*Radiation Therapy for PDH*  
Radiation therapy is recommended for cats with PDH that have macroadenomas causing neurologic side effects. Concurrent trilostane therapy can also be implemented. Improvement in neurologic signs may take weeks to months and signs associated with hypercortisolemia can also improve. Survival times of 1 to 2 years have been reported.

**Treatment of Iatrogenic Hyperglucorticoidism**  
When prescribing glucocorticoids for the management of any disease in the cat, it is important to be in contact with the pet owner and to reassess the cat for both desired clinical response but also the development of significant side effects from corticosteroid administration. If these develop,
then alternative drug options should be considered and glucocorticoids slowly tapered. The longer the glucocorticoid therapy the more slowly the tapering protocol should be.

**Prognosis**
Feline Cushing’s syndrome has a poor prognosis and it is often not diagnosed until the disease is advanced. As it occurs in older cats, there are often concurrent disease(s) present and owners may elect not to pursue any therapy for the HAC. Surgical options for treatment are most effective but have relatively high morbidity and mortality associated with them as these cats are not good surgical candidates. The goal of medical therapy with trilostane is to control clinical signs and facilitate management of the concurrent DM. Untreated, most cats are euthanized within a month of diagnosis. 

**References**
Autoimmune skin diseases are well-recognized but uncommon in cats.\textsuperscript{1} They have been reported to account for 1.3\% to 2.5\% of all feline dermatoses examined by the dermatology service at a university companion animal practice.\textsuperscript{2,3} A definitive diagnosis for some autoimmune dermatoses requires specific autoantibody demonstration, which is not usually available to clinicians in private and specialty practice. The recognized feline autoimmune skin diseases and some prevalence data are listed in Table 1 and Table 2, respectively.

Pemphigus foliaceus is, by far, the most common autoimmune skin disease in cats.\textsuperscript{1-4} When expressed as the percentage of the cats with dermatologic disease (1407 cats) and the percentage of all cats seen at the university hospital (22,135 cats) during the same time period, pemphigus foliaceus accounted for 1.2\% and <0.1\%, respectively, of the cats.\textsuperscript{3} Feline pemphigus foliaceus has no breed or sex predilections and occurs in cats 4 months to 17 (average 5) years of age. The distribution of skin lesions typically involves combination of the face, pinnae, neck, nasal plane, paws (especially pawpads and clawbeds), and ventrum (especially around the nipples). Pruritus varies from mild to severe. Systemic signs (fever, lethargy, poor appetite) are frequently present. Hematologic changes may include leukocytosis, neutrophilia, eosinophilia, basophilia, and anemia.

For most autoimmune dermatoses, skin biopsies and dermatohistopathologic findings are the key to accurate diagnosis and resultant therapeutic options and prognosis.

### Table 1: Autoimmune Skin Diseases in Cats\textsuperscript{1}

<table>
<thead>
<tr>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus foliaceus/erythematosus</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td>Discoid lupus erythematosus</td>
</tr>
<tr>
<td>Bullous pemphigoid</td>
</tr>
<tr>
<td>Mucous membrane pemphigoid</td>
</tr>
<tr>
<td>Cryoglobulinemia</td>
</tr>
<tr>
<td>Alopecia areata</td>
</tr>
<tr>
<td>Pseudopelade</td>
</tr>
<tr>
<td>Vitiligo</td>
</tr>
</tbody>
</table>
Table 2: Prevalence Data for Some Feline Autoimmune Dermatoses

<table>
<thead>
<tr>
<th>Dermatosis</th>
<th>% of Cats with Skin Disease</th>
<th>% of Total Cat Hospital Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pemphigus foliaceus</td>
<td>1.2</td>
<td>&gt;0.01</td>
</tr>
<tr>
<td>Alopecia areata</td>
<td>0.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Discoid lupus erythematosus</td>
<td>0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cryoglobulinemia</td>
<td>0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pseudopelade</td>
<td>0.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pemphigoid</td>
<td>0.1</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

References
Many dermatoses can cause pruritus in horses. Over one-fifth of the horses presented to dermatology services have allergic skin disease. This discussion will focus on the differential diagnosis of the “allergic-like” horse.

There are four classic allergic patterns in horses: (1) initially lesionless symmetrical pruritus (especially face, ears, neck, tail head, and/or distal limbs, (2) urticaria (especially neck, trunk), (3) sterile eosinophilic folliculitis (especially neck, trunk), and (4) eosinophilic granuloma (especially neck, trunk). Some horses have combinations of these patterns.

However, these cutaneous reaction patterns are also seen in nonallergic dermatoses (Table 1). Initially lesionless symmetrical pruritus may also be seen with various ecto- and endoparasitisms, and certain infections. Urticaria may be pruritic or nonpruritic and may be seen with a wide variety of conditions (Table 2). Sterile eosinophilic folliculitis must be differentiated from the more common infectious causes of folliculitis: bacterial (especially staphylococcal) infection, dermatophilosis, and dermatophytosis. Eosinophilic granuloma must be differentiated from other infectious and sterile granulomatous disorders, as well as certain neoplasms and cysts.

In addition to a thorough history and physical examination, laboratory evaluations (skin scrapings, tapings, combings, fecal flotations, trichography, cytology, fungal culture, skin biopsies), response to treatment (parasiticides), and avoidance (drugs, contactants, environmental niches) are often indicated to rule out nonallergic disorders. Only then should investigations that pursue allergies (novel diets, serological and intradermal “allergy” tests) be undertaken.

Table 1: Differential Diagnosis for Initially Lesionless Symmetrical Pruritus

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Lesionless Symmetrical Pruritus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatitis</td>
<td>Bird bites</td>
</tr>
<tr>
<td>Food allergy</td>
<td>Forage mites</td>
</tr>
<tr>
<td>Insect-bite allergy</td>
<td>Trombiculidiasis (chiggers)</td>
</tr>
<tr>
<td>Cutaneous adverse drug reaction</td>
<td>Oxyuriasis (still waiting!)</td>
</tr>
<tr>
<td>Psoroptic mange (early)</td>
<td>Intestinal parasite hypersensitivity (?)</td>
</tr>
<tr>
<td>Chorioptic mange (early)</td>
<td>Malassezia dermatitis</td>
</tr>
<tr>
<td>Sarcoptic mange (early)</td>
<td>Coital exanthema</td>
</tr>
<tr>
<td>Pediculosis (lice)</td>
<td></td>
</tr>
<tr>
<td>Louse-flies</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Differential Diagnosis for Urticaria

<table>
<thead>
<tr>
<th>Category</th>
<th>Differential Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cutaneous adverse drug reactions</td>
<td>Vasculitis (many causes)</td>
</tr>
<tr>
<td>Allergies (atopic, food, contact, drug)</td>
<td>Dermatographism</td>
</tr>
<tr>
<td>Plants</td>
<td>Cold</td>
</tr>
<tr>
<td>Insects/sarachnids</td>
<td>Exercise-induced</td>
</tr>
<tr>
<td>Snake bites</td>
<td>Cholinergic</td>
</tr>
<tr>
<td>Infections</td>
<td>Stress/psychogenic</td>
</tr>
<tr>
<td>Contactants</td>
<td>Idiopathy</td>
</tr>
</tbody>
</table>

Selected Reference
MANAGEMENT OF ALLERGIC HORSES

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Management of allergic horses requires identification and correction of the triggering cause together with successful management of the secondary infections, which complicate the clinical picture and significantly increase the level of pruritus.

A multimodal approach is crucial in most cases for maximum success. Many horses with atopic disease frequently have other allergic diseases (e.g., insect hypersensitivity). Therefore, the therapeutic approach needs to address all the components of the disease with the intent to get below a clinical threshold of pruritus. All factors are additive and each component may push the level of pruritus above the threshold of what can be tolerated by the specific patient. Thus, in the management of these cases, it is crucial to identify and manage all the components of the disease.

Many cases initially present with a secondary infection precipitated by the underlying allergy and self-trauma. Diagnosis of bacterial infection is typically made based on suggestive clinical signs (e.g., papules, crusting and scaling) and supported by cytology showing cocci and degenerated neutrophils. Depending on the severity of the infection, this can be either treated systemically or topically. Systemic antibiotic therapy frequently consists of oral administration of potentiated sulfonamides for 2 to 3 weeks. In the author’s experience, bacterial infections can become extremely severe to the point of voiding any benefit from glucocorticoids and other antipruritic agents. Horses reported to be highly pruritic and having failed treatment with Apoquel were completely controlled by addressing a resistant Staphylococcal infection and implementing aggressive insect control. Due to the increased resistance of Staphylococcus in horses, it is strongly encouraged to do culture and sensitivity in patients that show minimal improvement with sulfonamides and continue to show bacteria on cytology.

Topical therapy is useful as adjunctive therapy and may, in some cases, become the only option available. This involves use of medicated shampoos with benzoyl peroxide or chlorhexidine followed by conditioners to decrease pruritus (e.g., topical glucocorticoids, oatmeal, pramoxine). In some cases where shampooing is not feasible and systemic antibiotics are not desirable, topical application of oxychlorine spray (2 to 3 times per day) can be a very effective treatment. Another topical therapy that has been demonstrated to be effective is stannous fluoride (0.4%) and silver sulfadiazine for limited area of infection.

Many allergic horses have concurrent insect hypersensitivity and aggressive insect control is necessary. This is done by identifying risk factors and correcting them, as well as, modifying some aspects of the daily routine. Culicoides hypersensitivity is a common aggravating factor in atopic horses. Culicoides are weak flyers that do not cover long distances and preferentially feed from dusk to dawn. Vicinity to ponds, lakes or any area of standing water is a risk factor. Turn out at night is another risk factor. Moving horses to paddocks further away from the water and keeping the horses inside at night, while using strong fans, can significantly decrease exposure to Culicoides. It is also important to use insect repellents. Although many products are labeled as repellent very few have true repellent qualities. Preference should be given to products
containing 2% or higher of permethrin and to products that tend to adhere to the coat and not be easily washed off by the rain. Regardless of the product used, frequent reapplication is key, since even the best product does not have a long residual efficacy when horses sweat or in high humidity climates. Thus, daily reapplication is needed in the management of most cases of insect hypersensitivity. In the United States, many products are mislabeled as repellents when they do not contain active ingredients or concentrations which are effective. This is often confusing for owners. For this reason, it is very important to educate owners about the appropriate use of insect repellent products. Neem oil is a useful alternative for horses that cannot tolerate other chemicals.

If *food hypersensitivity* is part of the differential diagnosis, it is also important to control exposure to offending foods. This can be done by strictly avoiding those foods in the main diet as well as in any flavored supplement given to the animal. Knowledge of labels of the various commercial diets is essential. Common causes include hays rich in protein such as alfalfa or peanut. Soybean is also a frequent culprit and exposure needs to be monitored for possible worsening of clinical signs.

The most common equine allergy is *atopic dermatitis*, which in most cases involves allergies against environmental allergens such as grass, tree and weed pollens. This allergy can be addressed symptomatically with therapies aimed at decreasing the inflammatory response (e.g., antihistamines, glucocorticoids, pentoxifylline) and therapies aimed at modulating the allergic response by use of immunotherapy. For young individuals with a long season of clinical signs, immunotherapy is always preferable. Immunotherapy is formulated based on the results of allergy testing. Allergy testing can be done by various means ranging from serology testing to intradermal skin testing. Various studies have shown that positive results can also be obtained in clinically normal horses and that little correlation is found between intradermal skin testing and serology testing.\(^1\)-\(^3\) This emphasizes the importance of a diagnosis based on clinical signs and exclusion of concurrent diseases rather than based on an allergy test.

Very few controlled studies have been done on the efficacy of immunotherapy in horses with allergies and reported success rates have varied from 60 to 80\%.\(^4\)-\(^6\) As a general rule, most horses show improvement after the first 6 months of therapy and some even after the first 3 months. Immunotherapy should be continued for a full year to completely assess the benefit. It is advised not to include more than 10 to 12 allergens per vaccine and to prioritize the choice based on the patient’s history and correlation of the positive results with the patient’s environment and seasonality of clinical signs. Some patients with skin disease also have respiratory disease and immunotherapy can be beneficial to address both conditions. As immunotherapy takes months to be effective, it is important to also implement adjunctive therapy to make the patient comfortable.

Topical therapy is always beneficial although labor intensive. The benefit of topical therapy is to remove the allergens as well as to deliver antipruritic and soothing agents directly onto the skin. Good choices are typically topical glucocorticoids, oatmeal and pramoxine. These products can be used daily if needed.
In order to decrease pruritus, oral treatment with a combination of antihistamines and glucocorticoids can be useful. Very few published studies exist on the efficacy of antihistamines in horses with allergic disease. A recently published study reported no benefit with the use of cetirizine in horses with insect hypersensitivity. Other antihistamines commonly prescribed include hydroxyzine (1-2mg/kg q8-12hrs per os) and chlorpheniramine (0.25-0.5mg/kg q12hrs per os). It is important to note that antihistamines seem to work best with use before the beginning of allergy season and much less once the season has started. If needed, they can be combined with glucocorticoids. Prednisolone can be used at an induction dose or 2mg/kg q24hr for 3 to 10 days. Once the pruritus is controlled, this dose can be tapered to achieve 0.5mg/kg q48hrs. Some horses may require dexamethasone (0.2mg/kg q24hrs) although this is not as safe for long term management. Pentoxifylline can also be beneficial in some patients with allergies. This drug is well tolerated and safe for long term use. It can be used at 10mg/kg q8-12hrs.

One of the many manifestations of allergies in horses is urticaria. The causes for chronic urticaria in horses are numerous and part of the treatment of these frustrating cases is the identification and management of the triggering cause. For this reason, it is important to have a very thorough history to include information about exposure to drugs, vaccines, anthelmintics, supplements and feeds. In some cases, urticaria is triggered by cold temperature, water, exercise, physical trauma or pressure. Some cases have insect hypersensitivity and others have pollen allergies. Therefore, the most successful way to manage these cases is the identification of the triggering cause. For the cases in which an inciting cause cannot be identified, symptomatic treatment is the only option. In most cases, treatment involves the long term use of glucocorticoids since the use of antihistamines is typically of limited benefit. Hydroxyzine is probably the most helpful antihistamine. Pentoxifylline can also be tried due to its ability to stabilize mast cells.

In summary, the treatment of allergies in horses requires a comprehensive approach to identify and correct all possible contributing factors. The clinician should therefore be aware of the various contributors to the pruritus in an individual animal and attempt to either treat or control each of them. For individuals with long seasons of environmental allergies, immunotherapy remains the best long term option.

References

CULICOIDES HYPERSENSITIVITY – FUTURE PROSPECTS FOR DIAGNOSIS AND TREATMENT

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²Faculty of Medicine, University of Zurich, Zurich, Switzerland.

Introduction

Culicoides hypersensitivity (CH), also called insect bite hypersensitivity, summer eczema or sweet itch, is a T helper 2 (Th2)-type, IgE-mediated allergic dermatitis with an imbalance between T helper (Th)2, Th1 and regulatory T cells (Treg), usually accompanied by a late-phase reaction with eosinophil infiltration in the affected skin. CH is caused by bites of insects of the genus Culicoides (midges) and occurs worldwide with varying prevalence, except in Iceland, where the relevant Culicoides spp. are absent. While the prevalence of CH in horses of the Icelandic breed born in continental Europe or in the USA is similar to the overall prevalence in other breeds (8%), horses exported from Iceland to countries where these insects are present, have a high prevalence (>50% after two years following import) of CH.

Diagnosis of CH is mainly based on the typical clinical signs and the seasonality of the disease. While basophil activation tests have a satisfactory performance for confirmation of the clinical diagnosis of CH, commercially available serological test have been reported to have a low sensitivity and specificity. Accordingly, allergen immunotherapy (AIT) for CH seems to have a limited efficacy. This can be explained by the fact that whole body extract (WBE) of Culicoides spp. are used and that the relevant allergens are present in a too low concentration in these extracts. Furthermore, laboratory bred Culicoides species are used, which are not frequent in the environment of the horses. Thus, IgE serology as well as therapeutic options for CH are still unsatisfactory. Presently, the most effective treatments for CH are avoidance of insect bites through stabling, CH blankets or use of suitable repellents and symptomatic treatment with corticosteroids. Nevertheless, on-going research aiming at the production of Culicoides allergens as pure proteins, as well as studies exploring new possibilities for treatment and prevention of this disease may lead to an improvement of serological IgE diagnosis and treatment of CH.

Culicoides allergens

The allergens causing CH are salivary gland proteins that are injected into the skin during the blood meal of Culicoides midges. Because of the problems inherent with the use of crude Culicoides WBE and the difficulty to obtain Culicoides saliva, different research groups aimed at identifying and producing the specific allergens for CH using molecular technologies. Over the last ten years, about 30 Culicoides allergens have been characterized at the molecular level and produced as recombinant (r) proteins. The first recombinant Culicoides allergens that were identified and produced originated from Culicoides species that can be maintained in laboratory conditions (C. sonorensis, C. nubeculosus), but do not belong to the main species found in the environment of horses. Few years later, allergens from Culicoides species caught in the environment of horses were also identified and produced as r-proteins. Recent publications suggest that allergens derived from C. obsoletus, which are often the most frequent Culicoides spp. found in the environment of horses, are more immune-reactive for CH than those from
laboratory-bred, non-indigenous species. Availability of these pure *Culicoides* r-allergens is a prerequisite for development of improved *in vitro* diagnosis and AIT of CH.

**Improving IgE serology for diagnosis of Culicoides hypersensitivity using pure *Culicoides* recombinant allergens**

Various studies have shown that determination of serum IgE to *Culicoides* r-allergens by ELISA leads to test results with higher sensitivities and specificities than those described using a commercial test with *Culicoides* WBE. Due to the high number of different *Culicoides* r-allergens that must be tested, ELISAs are cumbersome and expensive. Pooling of different r-allergens in the same test is an attractive approach for a time and cost saving *in vitro* test to confirm clinical diagnosis of CH. Nevertheless, this does not allow component-resolved diagnostics (CRD); that is, defining to which specific r-allergen an individual is sensitised. CRD is necessary for the selection of the specific r-allergens to be included in AIT and is also useful to distinguish primary sensitization from cross-reactivity. Microarrays are a powerful tool to determine the sensitization profile of allergic individuals, as a large number of allergens can be tested in the same run using only a small amount of serum. A pilot study using a limited panel of *Culicoides* allergens together with a large number of (>300) allergen extracts and pure proteins derived from epithelium, pollen, fish, mollusca, fungi and insects has shown that this method in combination with mathematical modelling allows to discriminate CH-affected horses from healthy controls as well as from horses affected with other allergic conditions, such as recurrent urticarial or equine asthma. As expected, from all allergens tested, the group of *Culicoides* allergens was the major variable for discrimination of the CH horses group from the other groups. A new microarray with a nearly complete panel of 27 *Culicoides* r-allergens has now been developed and is being used for identification of the major *Culicoides* r-allergens that need to be included into a preventive vaccine against CH for horses imported from Iceland. A preliminary analysis showed that nine r-*C. obsoletus* allergens are major allergens for CH as > 50% of the CH-affected horses are positive with these allergens, while only few (≤5%) of the healthy control horses had positive IgE values. Consequently, these nine r-allergens are those that need to be included in a preventive vaccine for horses imported from Iceland to Europe.

**Allergen immunotherapy**

Allergen immunotherapy (AIT) is the only curative treatment of allergies. Furthermore, AIT can stop the “allergic march” (over time, acquisition of sensitisation to additional allergens and development of new allergic symptoms, such as allergic asthma after initial atopic dermatitis in human patients). Interestingly, recent studies indicate a similar phenomenon in horses. Unfortunately, the efficacy of AIT for CH is questionable, as a placebo-controlled study failed to demonstrate a significant improvement of clinical signs. The main immunological mechanisms underlying AIT are a shift of the immune response from Th2 towards a regulatory and/or Th1 response and the induction of IgG antibodies, in particular of so-called “blocking antibodies” (IgG antibodies which are able to block the binding of allergen-specific IgE to the allergens). Preventive AIT is increasingly being considered as an option to reduce the high prevalence of allergies in humans in western countries and a similar approach may be used to reduce the incidence of CH.
**Development of preventive allergen immunotherapy**

Since CH does not occur in Iceland and the prevalence of CH in Icelandic horses exported to *Culicoides* endemic area is high, Icelandic horses offer a unique opportunity for development and study of a preventive AIT. With the aim to develop a prophylactic AIT against equine CH, an immunization protocol was first established, with the aim to induce mainly a Th1/Treg immune response with production of IgG antibodies and, most importantly, without induction of an IgE response. A further aim was to induce a suitable immune response with relatively small amount of the r-allergens, as their production can be cumbersome and expensive. Application route and adjuvants were evaluated in different studies using horses living in Iceland (not exposed to *Culicoides* bites). These studies have shown that three immunizations into the submandibular lymph nodes with small amounts of *Culicoides* r-allergens (10 μg each) in Alum and MPLA (monophosphoryl lipid-A) used as adjuvants, promote a Th1/Treg response and induce a strong IgG antibody response. These IgG antibodies were able to block IgE-binding to the specific *Culicoides* r-allergens. This is a highly important feature of successful AIT. Indeed, no IgE antibodies were induced with this immunisation procedure and intradermal test performed at the end of these studies did also not indicate allergic sensitisation in the horses. To assess whether a preventive AIT can reduce the incidence of CH, 27 horses have now been vaccinated in Iceland with the nine most relevant *Culicoides* r-allergen using the protocol developed in the previous studies. These horses were exported to Switzerland in March 2020 and their clinical status will be monitored at regular intervals over 3 years.

**Active immunisation against interleukins as alternative to treatments using monoclonal antibodies**

*Active immunisation against equine interleukin-5*

Eosinophils are the dominant infiltrating cell type in CH skin lesions and blood eosinophil counts have been shown to correlate with CH lesion score severity. Interleukin-5 (IL-5) plays a central role for differentiation, activation and survival of eosinophils. In human beings suffering from hypereosinophilic conditions such as eosinophilic asthma, treatment with humanized monoclonal antibody against IL-5 leads to improvement of clinical signs, suggesting that a similar treatment could be beneficial for CH patients. This approach, however, will not be available for horses due to high production costs. A new approach has been developed. Horses were vaccinated with equine IL-5 linked to a virus-like particle (VLP) in order to outwit the immune system, which would usually not make an antibody response against a self-protein. Upon 3 injections of IL-5-VLP, a specific antibody response was established, which resulted in a decrease of blood eosinophil numbers and in a significant improvement of the CH lesion score in comparison to the placebo group. A yearly boost before the CH season further decreased the CH lesion scores in the following years, probably because of the more stable antibody responses with higher avidity. The treatment was safe (no side effects were recorded) and the degree of infection with endoparasites was not affected. Vaccination with IL-5-VLP thus represents a valuable approach for improvement of clinical signs in CH-affected patients.

*Active immunisation against equine interleukin-31*

A similar approach to the IL-5 vaccination has been developed using IL-31 as target. IL-31 is an important cytokine for pruritus and development of allergic diseases. Recent studies have shown increased mRNA expression of IL-31 and its receptors in the skin of CH-affected horses compared to non-affected control horses, indicating an important role of IL-31 in the
Pathogenesis of CH. A placebo controlled randomized double-blind study demonstrated a reduction of CH lesion scores in the vaccine over the placebo group. Further studies are now needed to investigate whether a combination of both IL-5 and IL-31 vaccines could further improve the clinical response.

Conclusion
Over the last decade, our knowledge about the specific causative allergens for CH has grown and different new therapeutic options for the treatment of this important disease of the horse are at a more or less advanced stage of development. Tools are now also available to implement an improved serological diagnosis of CH. Hopefully some of these new prospects will result in commercially available tests and treatments, allowing a better management of CH.

Selected References
Folliculitis and furunculosis are common cutaneous reaction patterns in the horse, accounting for 32% of the patients examined by a university dermatology service. The differential diagnosis of follicular dermatoses is lengthy (Table 1). The most common causes of equine folliculitis and furunculosis are infectious agents (Table 2).

**Folliculitis** is characterized by various combinations of superficial tufted papules, oozing, and annular crust/scale/alopecia. Pustules and epidermal collarettes are not commonly seen. Pruritus varies from nonexistent-to-marked. **Furunculosis** is characterized by various combinations of deep-seated papules, nodules, oozing, draining tracts, ulcers, and annular crusts/alopecia. Pruritus and pain are variable. Commonly affected cutaneous areas include the face, neck, trunk, and distal limbs.

In addition to a thorough history and physical examination, appropriate laboratory evaluations may include skin scrapings, cytology, trichography, culture, skin biopsies, and response to treatment.

### Table 1: Differential Diagnosis of Equine Hair Follicle Inflammation

<table>
<thead>
<tr>
<th>Infectious</th>
<th>Noninfectious</th>
<th>Mimics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcal dermatitis</td>
<td>Pemphigus foliaceus</td>
<td>Lupus erythematous</td>
</tr>
<tr>
<td>Dermatophilosis</td>
<td>Sterile eosinophilic folliculitis</td>
<td>Onchocerciasis</td>
</tr>
<tr>
<td>Dermatophytosis</td>
<td>Unilateral papular dermatosis</td>
<td>Sebaceous adenitis</td>
</tr>
<tr>
<td>Demodicosis</td>
<td>Alopecia areata</td>
<td>Follicular dysplasia</td>
</tr>
<tr>
<td><em>Pelodera</em> dermatitis</td>
<td>Linear alopecia</td>
<td>Occult sarcoid</td>
</tr>
</tbody>
</table>

1. References
Table 2: Prevalence of Causes of Equine Follicular Disease\textsuperscript{1}

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Percentage of Dermatology Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial follicular</td>
<td>11.8</td>
</tr>
<tr>
<td>Dermatophytosis</td>
<td>8.9</td>
</tr>
<tr>
<td>Dermatophilosis</td>
<td>5.6</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
<td>1.9</td>
</tr>
<tr>
<td>Alopecia areata</td>
<td>1.3</td>
</tr>
<tr>
<td>Follicular dysplasia</td>
<td>1.3</td>
</tr>
<tr>
<td>Unilateral papular dermatosis</td>
<td>1.2</td>
</tr>
<tr>
<td>Sterile eosinophilic folliculitis</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Selected Reference
1. Scott DW, Miller WH. *Equine Dermatology*. 2\textsuperscript{nd} ed. Elsevier Mosby, St. Louis, 2011.
Pastern dermatitis is a name used to describe clinical lesions that affect the lower legs of horses. It is not a specific diagnosis. Many diseases can induce lesions in this area and they are frequently lumped and described under this term. Slang terms for pastern dermatitis include scratches, dew poisoning, greasy heel, mud foot, mud fever, foot rot, and cracked heels. All these terms are descriptive of clinical presentations but have no diagnostic value.

When considering cases of pastern dermatitis it is important to consider predisposing causes, primary diseases and perpetuating factors. Predisposing causes increase the risk of development of disease such as thick feathers, extended periods in contact with wet grass or mud, or lack of pigment on the lower legs which can increase the risk of UV precipitated vasculitis. Primary diseases include those that are able to cause skin lesions in the pastern area on their own such as Culicoides hypersensitivity, contact allergy or mites. Perpetuating factors such as secondary infections aggravate the disease and prevent its resolution.

Regardless of the inciting cause, secondary infections are a very common complication and they can make the diagnostic and therapeutic process quite challenging. For this reason, it is important to have a systematic and logical approach for these cases. In the author’s clinical experience, the most common errors with pastern dermatitis are two-fold: first, cases are only treated with a variety of topical products, most of which are not able to penetrate in the skin (particularly in chronic cases), and secondly, failure to address the triggering cause. Too many times the emphasis is placed on the infections as they are the only problem, failing to realize that they will recur over and over unless the underlying cause is addressed.

Clinical approach to pastern dermatitis starts with a through history to include the age of onset, progression, seasonality and lifestyle of the patient. It is important to note response to previous therapies as well as the duration of treatment. It is important to know whether it was treated topically or systemically and which topical products have been used. As part of the history it is helpful to know whether pruritus was primary (e.g., evident at the beginning) or secondary (progressive over time once lesions and secondary infections have developed). Draft horses and horses with feathers on their lower legs are prone to the development of pastern dermatitis for a variety of reasons. Most horses have some level of pruritus and discomfort at the time of presentation. Some cases are mild and may only present with erythema, crusting and scaling, while other may develop significant crusting, oozing, edema and pain. The swelling can be so severe that lameness occurs. Draining tracts can form and granulation tissue formation can become significant. Lichenification and hyperpigmentation are evident in chronic cases. On physical exam it is important to note whether only the front limbs are affected or whether all four legs are affected. It is important to note whether only the depigmented limbs are involved or whether lesions are independent of pigmentation. It is also important to know whether there are multiple horses affected in the herd or just one.
Differential diagnoses for primary causes of pastern dermatitis include allergic (contact, food, insect allergy), parasitic (*Chorioptes*), bacterial (*Dermatophilus*), fungal (dermatophytes), immune-mediated (vasculitis), autoimmune (pemphigus foliaceus) and genetically inherited lymphatic disease (progressive lymphedema in draft breeds). Since the list is extensive it is important to address the secondary infections first (‘treat the treatable’) and reassess once the infections are resolved. It is important to address pastern dermatitis cases as early as possible; in chronic cases, it can be particularly difficult to diagnose the underlying cause.

Initial work up of these cases includes skin scraping, cytology and fungal culture. Frequently a bacterial culture is also recommended, particularly in chronic cases that have been treated with multiple courses of antibiotics. The best way to obtain a bacterial culture is by skin biopsy. Simply swabbing the skin or a draining tract may lead to culture of secondary contaminants, while missing the main pathogen. For this reason skin biopsy and culture of the tissue is the most appropriate way to culture these cases. *Staphylococcus* spp. is a very common cause of *secondary infection*. This is typically treated with systemic courses of potentiated sulfonamides although resistance is becoming increasingly common requiring bacterial cultures and sensitivity to identify suitable antibiotics. Systemic therapy in chronic cases may be needed for extended periods of time. Suitable topical antibacterial therapy includes antiseptics (chlorhexidine, oxychlorine) or antibiotics.

Depending on the results of initial tests, treatment for secondary infections and for *Chorioptes* may be initiated. *Chorioptes* can be treated topically with lime sulfur dips (once weekly for 3-5 times) and systemically with ivermectin (0.3mcg/kg q 2 weeks for 3 times) or moxidectin. Success of systemic therapy may not be complete due to the surface habits of this mite. All animals in contact with the affected one should be treated at the same time. Treatment should be extended pass the life cycle of the mite (4 weeks) with consideration the parasite can survive in the environment for several weeks. Before the dip, shampoo with an antibacterial product to help remove crusts (benzoyl peroxide). Fipronil has also been reported to be effective although this is an extra label use for this insecticide. It is also important to implement some general changes in management of these cases. If feather are present it is advisable to clip the legs to facilitate topical therapy and better visualize and clean the area. Also, if horses are kept in muddy and wet conditions, this should be modified to allow sufficient amount of time in dry paddocks or clean stalls.

Many topical treatments have typically been used in these cases. Thus, it is not uncommon for many patients to have also developed a contact dermatitis. Therefore, it is important to only use topical therapy that is needed and minimize the unnecessary use of topical products that may be irritating.

If contact allergy to plants or shavings is suspected, confinement can be done as part of the diagnostic plan. This can be done by applying bandages to the area after thorough cleaning of the skin or changing the life style of the animal for 7-10 days (if no infection is present). For example, if the horse is on pasture, it can be stalled and only turned out in a round pen where there is no grass. If the animal is primarily in a stall with shavings, different type of shavings can be used or turn-out can be done to rule out contact allergy. If a case has contact allergy and completely resolves with confinement, re-challenge will lead to recurrence of the lesions within
24-48 hours after exposure. Contact allergy is best managed with avoidance. If this is not feasible, then protective gear as well as oral pentoxifylline can be implemented. Pentoxifylline is dosed at 10mg/kg 3 times daily.

If infections have been treated, the next step can be biopsy for histopathology to identify the underlying disease. Vasculitis can be a cause of pastern dermatitis. This can be triggered by a variety of antigens or it can be idiopathic. Many cases are UV-triggered leukocytoclastic vasculitis that affects the white legs with increased frequency although it can be seen also in pigmented legs. Effort should be place in the identification of the triggering cause, if all possible. Careful review of drugs, dewormers and vaccinations should be done. The author has found that many of these horses are also allergic (atopic) and require allergen specific immunotherapy to decrease the frequency as well the severity of their flares besides symptomatic therapy for the already existing lesions. These cases present for crusted legs or punctate ulcers and frequently are reported for “stocking up” and even going lame. Diagnosis is based on clinical appearance and confirmed with a biopsy of an early lesion. Many of these horses initially require some glucocorticoid therapy to decrease the inflammation and swelling. The author always adds pentoxifylline as adjunctive treatment so that when the glucocorticoids are tapered and discontinued (typically in 2-3 weeks), the disease can be kept in remission with the use of pentoxifylline. Pentoxifylline has a lag phase of a couple of weeks and it is typically well tolerated. Another important component of the therapy is the UV avoidance for horses that have white legs. This can be accomplished by avoiding turning out the horse from 10am to 4pm, providing shaded paddocks and using elastic socks to protect the legs. Silver impregnated socks provide physical support to the limb vessels, antimicrobial effect from the silver and physical protection from direct UV light.

Food trials can also be considered in the event that the suspected trigger is an ingredient in the horse’s diet. Identification of a proper choice for food trial requires detailed history of hays and feeds used in the past. Many times commercially prepared pellets are temporarily discontinued as they have many common ingredients regardless of the brand such as soy and alfalfa. It is useful to identify a hay which is novel for the patient and supplement the hay with minerals and vitamins. For a food concentrate, oats and beet pulp may be added.

It is also important to avoid insect exposure as some species of Culicoides preferentially affect the lower legs of horses. Use of repellents such as 2% permethrin should be done daily in areas of great exposure.

In some draft horses (Shire, Clydesdale, and Belgian draft horses), a genetically inherited immune dysregulation leads to vasculitis and chronic progressive lymphedema. This condition is characterized by progressive swelling, hyperkeratosis, and fibrosis of the distal limbs. This condition is thought to have a genetic component and is chronic and progressive. The disease starts at an early age, progresses throughout the life of the horse, and often ends in disfigurement and disability of the limbs. Antibodies against elastin have been detected in affected horses. Horses with clinical signs of chronic progressive lymphedema have been found to have significantly higher anti-elastin antibody levels compared to clinically normal Belgian draught horses and to healthy warm-blood horses. These levels correlate with severity of lesions. These
antibodies could be used for early diagnosis of this condition and possibly to help with breeding programs to limit the breeding of individuals prone to this disease.

In summary, may different diseases can manifest as pastern dermatitis. Due to numerous possible underlying causes, a systematic and logical approach is crucial. History, careful physical exam and identification of primary lesions (when still present) are important in formulating the best diagnostic plan for each individual case. In most cases secondary infections are present and complicate the evaluations. Successful identification of the underlying disease will also depend on complete resolution of the secondary infections.

References
SKIN DISEASES IN DONKEYS

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Introduction
Donkeys (*Equus asinus*) are a species utilized throughout the world primarily as beasts of burden, but occasionally for other functions, as a meat source, as pets or use of their milk in cosmetics. While closely related to horses and zebras (with both of which they can produce sterile hybrids) they have some unique features of their own in regards to disease. These notes will briefly highlight some of the various dermatoses seen or reported in donkeys, as well as some comparisons to horses when prevalence, presentation or treatment may differ.

Vocabulary: in English, a male donkey is called a ‘jack’, a female is a ‘jenny’, and castrated male a ‘cut-jack’ or a ‘donkey gelding’.

Congenital Dermatoses
As has been reported in horses, junctional epidermal bullosa (JEB) has been noted in a donkey foal. The disease was characterized by ulceration of large areas of the skin on the legs and ears. Interestingly, the foal was noted to be ‘normal’ at birth. The progression of the disease is fatal.

Bacterial Disease
Perhaps the most important bacterial skin infection on a world-wide basis is that caused by the actinomycete *Dermatophilus congolensis*. Antibiotics, such as trimethoprim-sulfamethoxazole as well as topical washings are the recommended treatment. 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. Thus, dosing intervals probably need to be more frequent in donkeys than in horses. The same has been found in preliminary research for amikacin, oxytetracycline, and the beta-lactam antibiotics. The opposite seems to be the case for the fluoroquinolone marbofloxacin. Another antibiotic in the same class, norfloxacin should probably be avoided in donkeys, because of neurologic signs when injected intravenously, swelling at intramuscular injection sites, and poor bioavailability when given orally.

Other bacterial skin infections, such as those caused by staphylococcal species or *Corynebacterium pseudotuberculosis* are also occasionally seen in donkeys.

Fungal Infections
*Trichophyton verrucosum* and *T mentagrophytes* have been reported as causing alopecia and scaling in donkeys. Diagnosis is by fungal culture and/or histopathology.

Deeper fungal infections, such as those caused by *Sporothrix schenckii*, *Cryptococcus* spp, *Histoplasma capsulatum* (North American histoplasmosis), and *Histoplasma capsulatum* var *farcininosum* (*‘farcy’ — the cause of equine epizootic lymphangitis in east Africa*) have been reported in donkeys. The latter fungus has yielded a number of articles on both clinical signs and diagnoses in horses. In general, deep fungal infections present clinically with nodules and ulcers,
often with a purulent exudate. Sporotrichosis seems to have a tendency to affect the inguinal area and medial aspect of the rear legs although other areas of the body may also be affected. Thickened (‘corded’) lymphatics may be noted, especially in *Histoplasma capsulatum var farciminosum* infections. Diagnosis may be achieved by cytology, culture, histopathology or, in some cases, by direct immunofluorescence of the biopsy tissue.

Treatment with oral potassium iodide (KI) and fluconazole may be helpful in controlling, if not curing, these deep fungal infections. Reported dosage regimens for KI are 0.5-2 mg/kg daily for at least 2.5 years or 40 mg/kg daily for 10 days; dose for fluconazole is 1 mg/kg daily for at least 2 years.

**Viral Infections**
Molluscum contagiosum has been reported in donkeys. Like horses, donkeys are susceptible to the mucosal viruses, such as vesicular stomatitis.

**Parasitic Infestations**
*Habronema* spp, lice, biting flies and *Chorioptes* spp can all afflict donkeys as they do horses. Anecdotally, cutaneous habronemiasis has been thought to cause more severe lesions (in general) in donkeys and mules than in horses. Donkeys can be afflicted with a hypersensitivity to *Culicoides* spp.

Also similar to horses, donkeys may be infested by biting lice (*Werneckiella equi*); the distribution over the body may be more widespread than in horses. A study from Italy demonstrated efficacy of the insecticide alphacypermethrin after one dose of 0.5% pour-on at the dose of 10 mL per donkey.

A form of cutaneous onchocerciasis is seen in donkeys in Africa and possibly in other nearby areas, which causes severe ulceration in the withers and neck region of donkeys; it is the adult parasite (sometimes extractable by hand) that causes this problem. The possible causative species is *Onchocerca raiilieti*. Interestingly, ivermectin has been reported as having both greater and lesser gastrointestinal absorption in donkeys than in horses. This awaits more clarifying studies.

Donkeys are susceptible to infestation with the parasite *Besnoitia bennetti*. This parasite has been reported from donkeys in the USA, Africa, and Europe. This organism is the same species which afflicts cattle, and is closely related to *B. tarandi* which afflicts reindeer (*Rangifer tarandus*). The disease presents as nodules (cysts) on the skin, nares, and sclera. Scaling on the skin may be noted. Multiple donkeys in a herd may be affected. Diagnosis may be by histopathology, serologic testing, and PCR. Unfortunately there is no known effective treatment.

*Pythium insidiosum* is the organism responsible for a severe cutaneous and subcutaneous disease seen most commonly in tropical and subtropical areas of the world. In the USA, it is most often found along the Gulf coast, southeast, and Hawai’i (although the author has seen a case in a horse that never left northern California). *P. insidiosum* is not a bacteria, fungus or parasite, but is in its own phylum, which is why it is very difficult to treat with available medications. It is a water-borne organism, thus most lesions are on the legs, face or ventrum (where the animal may be in contact with water while feeding). Lesions are ulcerative, proliferative and exudative and
are sometimes referred to as ‘swamp cancer’. There is one report of a donkey in Brazil whose larger lesions were excised and the smaller ones resolved spontaneously.

**Neoplasia**
Sarcoids are the most common cutaneous tumor in donkeys. Donkeys (particularly male donkeys) have a higher incidence than horses of sarcoids. As in horses and zebras, a number of researchers have been able to isolate bovine papilloma virus DNA from sarcoids of donkeys. Successful treatment with surgery, cisplatin, or both modalities has been reported.

**Auto-Immune Disease**
Donkeys have been described with pemphigus foliaceus, wherein the donkey’s own antibodies attack the intercellular proteins connecting the epidermal cells. In one jenny, the disease occurred during, then regressed after, 2 of its 5 pregnancies.

There is a report of a donkey with alopecia areata, where the donkey’s lymphocytes attack the hair follicle, causing wide spread hair loss.

Equine sarcoidosis (aka sterile granulomatous disease) has been seen in at least one donkey (Dr. Wayne Rosenkrantz, personal communication, Anaheim, California, December 2012). The pathogenesis of the disease is unknown, but possibly is an over-reaction by the immune system against an unknown pathogen or self-protein.

**Miscellaneous Diseases**
Donkeys have been reported with pituitary pars intermedia dysfunction (PPID), also known as equine Cushing’s disease. As in horses, donkeys with PPID can present with excessively thick and long coat. The initial dosage of pergolide reported as effective in treatment is 0.02 mg/kg.

‘Seedy toe’ is by definition an abnormal separation at the white line into which foreign material frequently gets wedged and forced into the depth of the hoof wall. Infection is common. It can arise from laminitic or overgrowth or management problems and is usually regarded as a preventable disease entity.” (Professor Derek Knottenbelt, personal communication via e-mail, January 2012). Based on research done at the Donkey Sanctuary (www.thedonkeysanctuary.org.uk), the donkey hoof wall is capable of much greater absorption of water than a horse — presumably an advantage in retaining scarce water in the arid environment where donkeys evolved, but a detriment in moist climates or wet stables such that the hooves may be prone to the keratolytic process of white line disease (personal communication via e-mail, Dr. Alex Thiemann, October, 2011).

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SKIN DISEASES OF SMALL MAMMALS

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SARCOPTIC MANGE IN WOMBATS AND KOALAS
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Sarcoptic mange disease (or scabies in human beings) is caused by the parasitic mite Sarcoptes scabiei and has been documented infecting/infesting mammals worldwide. The parasite has a particularly fascinating history, likely first documented in Leviticus in the Bible (around 1200 BCE) as a skin disease of human beings and other animals (Arlian and Morgan 2017). The parasite is also believed to be the first documented ‘definitively known cause of human disease’ in the history of human medicine, dating back to 1687 (Arlian and Morgan 2017). It was formally described and named Acarus scabiei DeGree in 1778 and reclassified Sarcoptes scabiei Latreille in 1802 (Arlian and Morgan 2017). Today, S. scabiei is known to infect more than 100 mammal species worldwide and the host range is still expanding (Pence and Ueckermann 2002b, Tompkins et al. 2015). Sarcoptic mange also remains one of the 50 most common human diseases (point prevalence, 100 million cases) and was recently labelled by the World Health Organisation a Neglected Tropical Disease of humans (WHO). Combined, Sarcoptes scabiei is among the most host generalist and burdensome of mammalian parasites (Pence and Ueckermann 2002, Vos et al. 2013).

The mite infests the epidermis (stratum corneum) of its host and host species vary in the severity of disease caused by S. scabiei. Nevertheless, the general pathology caused by S. scabiei is broadly similar across host species (Pence and Ueckermann 2002, Leung et al. 2020). Infection results in a type IV hypersensitivity reaction, which leads to skin inflammation and other more severe symptoms, including alopecia, hyperkeratosis, pruritus and emaciation (Bornstein et al. 2001, Pence and Ueckermann 2002, Skerratt 2003). Immunocompetent hosts (such as healthy people) experience mild skin inflammation as mites are killed by the immune system. The infestation of less competent hosts (e.g., canids) typically leads to an inflammatory immune pathway, more severe symptoms and discomfort to the host, but few mites detectable on or in the epidermis (Peltier et al. 2018, Niedringhaus et al. 2019). Highly susceptible and immunocompromised hosts (e.g., wombats, koalas and immunocompromised human beings) experience the most severe form of mange disease, crusted mange, characterised by an anti-inflammatory immune response, emaciation, significant hyperkeratosis and fissures, secondary infections and mortality (Pence et al. 1983, Bornstein et al. 2001, Martin et al. 2018b).

Koalas and wombats are among Australia’s most iconic wildlife and both of these marsupials are impacted by S. scabiei (Martin et al. 2017). Sarcoptic mange is the most important disease of the bare-nosed wombat (Vombatus ursinus, also known as, common wombat) and among the most important pathogens of the koala (Phascolarctos cinereus) (Martin et al. 2017). It is widely held that S. scabiei was introduced to Australia by European settlers and their domestic animals. The first reports date back to the 1820s (people and dogs) (Plomley 2008) and it is likely wombats were exposed around this time or earlier. The first documented outbreak of mange was in wombats in the mid-1930s (Gray 1937, Skerratt et al. 1998). While there is a reasonable body of mange in wombats, knowledge from koalas is relatively sparse. Mange is widespread across the geographic range of the bare-nosed wombat (eastern New South Wales to Tasmania and to eastern South Australia (Martin et al. 1998)), persisting endemically with periodic epizootics causing localized population decline and extirpation events (Skerratt 2005, Martin et al. 2018a).
In koalas, mange is documented in South Australia and Victoria (Speight et al. 2017). The disease is widely held as an animal welfare issue (owing to prolonged and severe disease) and an occasional conservation issue (when causing population declines in wombats) (Martin et al. 2017). However, the base-line impacts of mange on wombat or koala populations are unknown (Beeton et al. 2019). Transmission of *S. scabiei* appears environmental for wombats, with exposure to environmental fomite believed to occur in burrow chambers and intra-individual transmission events associated with periodic burrow switching behavior (Martin et al. 2018a, Martin et al. 2019). Transmission to koalas is poorly understood, but may occur when climbing down trees that a scabietic canid (e.g., foxes, wild dogs and dingos) has scratched itself against (Fraser et al. 2016, Speight et al. 2017).

Beyond direct epidermal impacts, knowledge of the individual level consequences of increasing sarcoptic mange severity mainly come from wombats, rather than koalas. At the physiological level, research has shown that mange disease results in up increased loss of heat energy to the environment owing to alopecia (Simpson et al. 2016, Martin et al. 2018b). There is also a marked increase in metabolic demand, likely due to investment in the immunological response (Martin et al. 2018b). Emaciation also increases with disease severity and the constituents of subcutaneous fat also change, indicative of the immunological investment (Simpson et al. 2016, Martin et al. 2018b). These physiological observations interplay with behavioral effects and what appears to be feelings of discomfort, including increased periods of foraging, decreased foraging efficiency and disrupted sleep (Simpson et al. 2016, Martin et al. 2018b). Collectively, physiological and behavioral research indicates wombats attempt to forage more to meet the increased metabolic demands of the disease unsuccessfully. They experience significant discomfort ultimately culminating in mortality of the weakened individual from an opportunistic bacterial infection. It is likely koalas experience similar physiological and behavioral impacts.

Sarcoptic mange is unusual in that the disease is visible. Observations of sick and dying wombats and koalas by the general public occur and there is increasing public pressure and action to find solutions to the problem. Presently many wildlife carers treat wombats *in situ* with pour-on ivermectin or moxidectin (Rowe et al. 2019). The drugs are applied directly by a ‘pole and scoop’ method or indirectly via a ‘burrow flap with treatment reservoir’ method (Old et al. 2017). Research suggests wombats metabolise ivermectin and moxidectin in one week or less, and *S. scabiei* mites can potentially survive in a wombat burrows for up the three weeks (Martin et al. 2019). Accordingly, ‘best practice’ guidelines for treating wombats for sarcoptic mange are weekly drug administrations for at least eight weeks, followed by fortnightly administrations out to 16 weeks. This treatment regime presents significant logistical challenges and the success of treatments by the public are often unknown and likely unsuccessful (Martin et al. 2017). Additionally, drug concentrations administered by the public are also highly variable, leading to other ethical considerations. More recently, there has been research investment into testing the control of sarcoptic mange at individual and population scales (Martin et al. 2019). The outcomes demonstrate that with enough effort, control is possible, but the logistical constraints are high (Martin et al. 2019). Key challenges identified have included successful treatment delivery and the duration protection conveyed by treatments. To address these gaps there are trials currently underway testing the safety, pharmacokinetics and efficacy of fluralaner for wombats, with promising early results.
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COMMON DERMATOLOGIC DISORDERS IN PET BIRDS

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Introduction

Although feather destructive behavior (also referred to as feather-picking or feather plucking) is the most common dermatological complaint seen in pet psittacine birds, there are many other causes of avian skin and feather disease encountered in practice. A full understanding of the structure and function of avian skin and feathers and of these other diseases is necessary as part of the logical diagnostic approach and rule-outs in any case of feather destructive behavior. It should be appreciated that some diseases may or may not be seen in conjunction with feather destructive behavior.

Avian skin anatomy

Avian skin is relatively thin with the epidermis consisting of only 2-10 cell layers in most areas except the feet which are covered with shield-like plates of keratin named scutes. The dermis has a rich blood supply especially in more specialized skin areas such as the wattles. Beneath the dermis is elastic tissue and connective tissue, which allows movement and attachment of underlying structures, but compared to mammalian skin is quite closely attached to these, making it relatively immobile.

Adnexal structures

The uropygial or preen gland is bilobed gland that lies dorsally near the tip of the tail and secretes lipoid sebaceous material that is spread across the plumage during preening. The size varies across species and the gland is absent in certain species such as Amazon parrots and Pionus parrots. There are no sweat glands; instead heat is lost via the respiratory tract and by radiation from featherless areas.

Beak conformation varies amongst the different bird species. The outer layer of the beak is formed by a thick stratum corneum. The dermis in this location contains collagen and elastin fibres and is closely attached to the periosteum that blends into the bone.

Feathers

Feathers comprise keratinized epidermis derived from follicles in the dermis and function not only to facilitate flight but also have a function in relation to insulation, waterproofing, courtship and camouflage. In most species, the feathers are set in feather tracts known as pterylae, and the featherless tracts between them are known as apterylae. These bare tracts can easily be mistaken as lesions or lacerations as the skin is so thin, but they are also useful for visualization of underlying structures such as the jugular vein, without the need for plucking feathers. There are various types of feathers with different appearances and functions including flight (primaries and secondaries) and tail feathers, contour feathers, down feathers, powder downs, bristle feathers and filoplumes. The basic structure of a flight feather is a hollow shaft (rachis) and base (calamus). The feather vane is comprised of filaments called barbs which extend on either side of the rachis. The barbs are zipper together by rows of small hooks called barbules.
New feathers developing in the dermal papilla force the shedding of old feathers above it. The shedding and replacement of feathers occurs at least once a year, usually after breeding, but can occur more frequent. The duration varies between species and between wild and captive populations. The moult starts with the inner primary feathers, followed by outer primary feathers and secondary feathers. Birds in temperate zones moult prior to the change of seasons while tropical birds moult gradually and continually. Thyroid hormones are important in normal feather development but are not a stimulus for moult. Changing of photoperiod and temperature are presumed to influence hormonal factors and induce moult.

Factors affecting moult:
- Nutritional deficits such as hypovitaminosis A
- Reduced protein levels result in poor feather condition and 'fret marks'.
- Stress or fear can induce a stress moult.
- Reduction to shorter periods of light (especially after prolonged periods of light) induces moult (through stimulation of the pituitary gland).
- Ectoparasites can cause abnormal moult patterns.
- Hypothyroidism leads to retardation of feather growth.

Viral skin disease
*PBFD (Psittacine beak and Feather Disease)*. Psittacine beak and feather disease is an important viral infection of mainly Old World parrots with only few clinical cases being documented in New World Species. Some birds, especially rainbow lorikeets (*Trichoglossus haematodus*), can be latent carriers shedding virus while appearing clinically normal. PBFD is caused by a DNA circovirus (BFDV) that can remain stable in the environment for up to a year. Formerly, it was believed that the circoviruses recovered from a diverse range of psittacines were all antigenically similar, but more recent research indicates that psittacine circoviruses can be divided into two species (beak and feather disease virus, BFDV; budgerigar circovirus, BCV) and multiple viral strains. The strain affecting lorikeets can appear to be less pathogenic and apparent recovery is possible, but they are still shedding virus and may often relapse. The PBFD virus is shed in feather dander and feces and is transmitted by inhalation or ingestion. Vertical transmission is also possible. The virus favors rapidly dividing cells and the clinical signs are related to its effects on dividing tissues and the targeted systems are the epithelial cells, gastrointestinal tract and immune system. In essence, three forms of PBFD can occur:

1. Peracute disease, which is mainly seen in neonates. Affected birds show signs of septicemia, pneumonia, enteritis, rapid weight loss and death.
2. Acute disease, which is usually seen in young or fledgling birds during their first feather formation and results in death in one to two weeks. This form is commonly seen in cockatoo and African grey parrot nestlings and is characterized by depression, diarrhea, crop stasis and death. These birds can also develop anemia and a (severe) leukopenia. Birds will often die before feather abnormalities develop.
3. Chronic, or ‘classic’ PBFD, which usually occurs in birds aged six to 12 months undergoing their first adult moult but can also be seen in older individuals. Death generally occurs six months to two years after the onset of clinical signs due to the immunosuppressive nature of the infection. Incubation period can be as short as three weeks or as long as twelve months.
Diagnosis is based on a PCR for the virus, which is commercially available. This can be taken from pulpy feathers which can be gently plucked or from a blood sample. The virus is present in the leucocytes; severely leucopenic birds may therefore have a false negative blood test. If suspicious of PBFD but the test result is negative test then a feather follicle or bone marrow biopsy can be taken for PCR. In case of a deceased bird, histopathology of the bursa of Fabricius (dorsal to the cloaca), bone marrow or skin may reveal the basophilic intracytoplasmic inclusions or lymphoid depletion typical of this infection. Any suspect carcasses should be kept frozen as a PCR can be performed on frozen tissue. Positive birds that are clinically healthy should be quarantined and re-tested in 90 days to see if they have cleared the virus.

Treatment is supportive, and may include treatment for secondary pathogens such as Aspergillus and Chlamydia. Some work using avian interferon has suggested an increased survival rate but the product is not available commercially and mammalian interferon was found to be of no use. Control is through proper quarantine and biosecurity. Thorough screening is recommended prior to allowing a new bird to enter a collection or a new home. The virus is extremely stable in the environment. The only disinfectant that has been shown to be effective is the peroxygen compound, Virkon-S, if it contacts the virus for a minimum of 10 minutes.

Avian Polyoma virus (Budgerigar fledgling disease). Avian Polyoma virus (APV) is a DNA papovavirus that affects all parrots and passerines. Typically budgerigars are clinically affected but the virus may also cause morbidity and mortality in other psittacines, especially very young macaws, eclectus parrots and conures. Caiques, ring-necked parakeets and lovebirds may be older, up to 1 year of age, when affected. APV can also cause mortality in nestling passerines such as Gouldian finches. The virus is shed in the feces, feather dust and secretions. Transmission is via inhalation or ingestion. Vertical transmission may be possible, but transmission to chicks can be horizontal as the virus is shed in crop secretions.

Clinical signs in budgerigars include neonatal death (can have abdominal distension, subcutaneous hemorrhage and reduced down and contour feathers). If neonates survive greater than 15 days, they will lose tail and flight feathers and are known as ‘French moulters’. PBFD can cause similar signs and concurrent infection of budgerigars with both avian polyomavirus and PBFD have been reported. If they survive, the feather loss resolves after several months. Recovered birds are carriers and shed when stressed. In other parrots, sudden death occurs or death after depression, weight loss, diarrhoea, regurgitation, subcutaneous haemorrhage, dyspnoea and polyuria. A chronic form manifests as weight loss, intermittent anorexia, polyuria, recurrent secondary infections, poor feather formation and neurological disease. In passerines acute death in fledglings and adults can occur. Beak abnormalities and feather dystrophies may also be present.

The diagnosis is made by PCR on blood or cloacal swabs and to some extent clinically. The psittacine PCR does not work in finches. On post mortem examination there is hepatic necrosis, bursal lymphoid depletion, membranous glomerulopathy, basophilic intranuclear and intracytoplasmic inclusion bodies in feather follicles and renal tissue. Control is dependent on good quarantine with closed flocks. In budgerigars, the recommendation is to stop breeding and clean out facilities for 3 to 6 months. In other parrots identifying shedders and removing them may be economically feasible. There is a vaccine in the USA.
**Poxvirus.** Most poxviruses are species specific and transmitted by biting insects. Wet pox (diphtheroid form) occurs in mucosa (oral cavity and trachea). An acute septicemic form also exists. Cutaneous or dry pox causes nodules, papules or vesicles on the skin. The cutaneous or dry pox is seen more commonly in raptors and songbirds as nodular lesions on feet, eyes or face. Canaries and finches develop lesions on the face, mouth and feet and will often develop severe pulmonary complications. Diagnosis is through histology or impression smears (intracytoplasmatic eosinophilic inclusion bodies or Bollinger bodies). Infection is generally self-limiting (3-4 weeks) and treatment will include supportive care and antibiotics for secondary bacterial infection. A vaccine is available for pigeons and canaries. Control of insects is also important in prevention of the disease.

**Papilloma virus.** Papilloma virus is associated with benign epithelial tumors. Therapy depends on their location (cloaca, oral cavity or skin). Papillomas may reoccur after removal and spontaneous remission has also been described.

**Parasitic skin disease**

*Mites.* Mites include *Cnemidocoptes* that causes scaly legs and face. These mites are normally located on featherless areas and their burrowing activity stimulates hyperplasia and hyperkeratosis. They are most commonly seen in budgerigars where they typically cause beak deformities, but can also be frequently diagnosed in backyard poultry and canaries where they result in “chalky legs”. The mites spend their entire life cycle on the bird and are transmitted from bird to bird. *Dermanyssus* spp. (red mites) are usually visible at night on the bird and spend the day free living in the cage or nest, in contrast to *Ornithonyssus sylviarum* (northern fowl mite) that is present on birds continuously. They are primarily bloodsucking mites causing anemia and death in fledglings. A white sheet placed over the cage at night will enable to visualize the mites the following morning. The mites can get on to humans causing irritation. Several species of feather and quill mites are known to inhabit the quill or vane of the feathers. When present in large numbers, they usually lead to irritation, pruritus, poor feather quality and feather destructive behaviour.

*Lice.* Lice may cause feather damage and irritation if in high numbers. This occurs when the bird is debilitated or due to poor husbandry. Lice appear to be host specific and cluster in a specific area of the body. The complete life cycle occurs on the host.

*Giardia/Spironucleus.* *Giardia* and *Spironucleus* are endoparasitic protozoal organisms that have been associated with feather destructive behavior in cockatiels and budgerigars. While the exact pathogenesis remains unknown, possible explanations include a hypersensitivity, or possibly a malabsorption/nutritional effect. A fresh direct fecal smear can be examined for trophozoites. Metronidazole at 30mg/kg PO once daily for 7-10 days is the treatment of choice.

**Bacterial and fungal skin disease**

Primary bacterial and fungal disease of the skin is less common. Bacteria isolated from skin infection in birds include *Staphylococcus, Mycobacterium, Mycoplasma* and *Aeromonas* species. *Candida* has been associated with hypovitaminosis-A. *Microsporum, Trichophyton, Cryptococcus neoformans* and *Malassezia* infections have been reported.
Superficial cutaneous ulcerative dermatitis (SCUD). SCUD is commonly seen in lovebirds but may be seen in other psittacine species as well. The exact etiology is unknown. Self mutilation is commonly reported, with factors such as poor nutrition, Giardiasis, Agapornis poxvirus, BFDV, polyoma and/or bacterial infections contributing to the problem. Affected birds usually mutilate along the patagial membrane, axilla, shoulder and neck. Commonly cultured bacteria include Enterobacter cloacae, Escherichia coli and Staphylococcus aureus. Diagnostic work-up may include a dietary history, hematology and biochemistry and screening for avian viruses. A swab for bacterial culture and skin biopsy can be taken from the lesions. Treatment depends on the diagnosis. Surgical debridement and closure may be indicated if the wounds do not heal with supportive care. A collar may have to be placed concurrent with therapy, which may frequently become part of the long-term management of these cases.

Nutritional factors in skin disease
Chronic malnutrition commonly presents as deterioration in the integument and plumage when malnutrition has reached severe levels. Hypovitaminosis A affects moulting and causes hyperkeratosis and glandular metaplasia of the uropygial gland. Reduced protein levels result in poor feather condition and 'fret marks'. Lysine, methionine and cysteine are essential amino acids necessary for feather quality, growth and pigmentation, but are deficient in all-seed diets. Nutritional related feather changes are potentially reversible but owners should always be advised that improvements will take up to 18 months as old feathers need to be moulted first.

Hypothyroidism
Hypothyroidism leads to retardation of feather growth, obesity and hyperlipidemia. True hypothyroidism is often misdiagnosed and a low blood thyroid level is not definitive for a diagnosis in pet birds. Thyroid hormone levels fluctuate throughout the day and many general disease conditions can also lead to lower circulating blood thyroid levels. A positive response to thyroxine is also not a definitive diagnosis, which necessitates a TSH stimulation test. The use of the TSH stimulation test has been reported in parrots at 1.0 IU/kg IM whereby a repeat blood sample is taken 6 hours later for T4 level, which should have at least doubled by then in euthyroid birds. In case of confirmation of hypothyroidism, L-thyroxine at a dose of 0.02mg/kg orally once or twice daily can be used.

Brown cere hypertrophy
This condition is typically seen in older female budgerigars with hyperplasia of the cornified layer of the cere, or in males with testicular tumors. Intervention is not required unless it is growing towards the skull or occluding the nares. Hypertrophied material can be scraped away. In male budgerigars with testicular tumors, the mass effect in the coelom can lead to dyspnea, and unilateral paresis/paralysis. In these cases, surgical removal of the tumour or placement of a deslorelin (GnRH analogue) hormone implant can be considered.

Cutaneous growths
Cysts. Follicular cysts are commonly seen in canaries with keratinaceous debris accumulating in an occluded feather follicle or a deformed feather within a follicle. They are now considered to be basal cell tumors or feather folliculomas (a benign neoplasm). Feather follicle cysts occur most commonly on the wing and along the chest or back. Treatment of a small isolated lesion is
not immediately warranted. However, if there are multiple lesions, or if the lesion is causing discomfort or hindrance to the bird, it can be surgically removed using an electrosurgery unit. Feather cysts are likely to re-occur.

*Squamous cell carcinoma*. These are common malignant tumors seen on the skin and oral cavity of various psittacine species including budgerigars and Amazon parrots. They can be locally invasive and need surgical removal. Cryosurgery can be used.

*Polyfolliculitis*. Polifolliculitis is a term used to describe the condition where multiple feather shafts appear from the same follicle. While generally no systemic effects are noted, the condition may lead to irritation and feather destructive behavior or self mutilation. The cause is not known but one should screen for known aetiologies for skin and feather disorders (e.g., BFDV, polyomavirus).

*Lipoma*. Lipomas are benign proliferation of lipocytes commonly seen in budgerigars in the subcutis of the sternum and abdominal skin. Obesity and advancing age predispose birds to developing lipomas. The bird should be placed on an appropriate diet and allowed plenty of exercise.

*Xanthoma*. Xanthomas are locally invasive benign masses consistent of foamy macrophages, multinucleated giant cells and cholesterol crystals. They are common in budgerigars and to a lesser degree in cockatiels. Yellow friable tissue is distributed over dorsal areas of the bird or over underlying conditions. Feed a low fat and low protein diet when possible. Some xanthomas are thyroid or L-carnitine responsive (0.1mg tablet L-thyroxine in 120ml drinking water; 1 g of L-carnitine in 1 kg of food).

**Foot diseases**

*Constricted toe syndrome*. This occurs when an encircling fibrous scab or necrotic tissue inducing an avascular necrosis of the distal extremity. The etiology is unknown and possible causes include low humidity and fiber-related strictures. To relieve the stricture, multiple incisions at right angles are made over the fibrous stricture.

*Bumblefoot*. Bumble foot or pododermatitis can be classified into five grades according to the changes in appearance of the plantar surface of the foot (metatarsal pad and digital pads). Causes are usually multifactorial and can include trauma, chronic wear, pressure or abrasions due to poor substrate, inadequate perches, lack of exercise, increased weight bearing due to medical conditions, malnutrition and/or obesity. Treatment will depend on the grade of bumble foot with husbandry changes often being sufficient for mild cases and surgery being necessary for severe cases. Corrective husbandry may include treatment of underlying medical conditions, dietary corrections, ensuring appropriately sized and types of perches and/or substrates, reducing the bird's weight and stimulating exercise. Foot soaks with dilute chlorhexidine or povidine-iodine, bandaging, antibiotics (based on culture and sensitivity) and anti-inflammatories may be indicated when moderate lesions are present, whereas debridement of infected and/or necrotic tissue under general anaesthesia will be needed in the more advanced cases. In such cases, radiographs should be considered to assess joints and bones, which may become involved in the disease process as well, leading to a more guarded to even poor prognosis.
Selected References
SKIN DISEASES OF REPTILES AND AMPHIBIANS

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*No published proceedings for this presentation—this page left blank for taking notes*
LEPROSY IN EURASIAN RED SQUIRRELS

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Introduction

Leprosy is an ancient chronic infectious disease of human beings, with descriptions dating back to 1500 BC. It is likely that the disease spread along ancient trade routes across Europe where the height of human infections occurred in the 12th to 15th century. Leprosy was introduced to the Americas with European colonialists and the slave trade. However, leprosy is by no means a disease of the past, and continues to be a public health focus and a challenge to researchers. Globally, more than 200,000 new human leprosy cases are detected annually, most of these in Southeast Asia. This is despite leprosy having a reputation as being the “least infectious of all infectious diseases” and an estimate that 90-95% of the human population will not develop clinical disease even when exposed to either of the causative agents, Mycobacterium leprae and Mycobacterium lepromatosis. While M. leprae was already identified as causative agent of leprosy in 1873 by Gerhard Hansen, M. lepromatosis was first described in 2008. Both Mycobacteria can occur separately or as coinfections, and can often only be differentiated by genetic sequencing. The clinical and histological presentations of leprosy in people are not uniform but fall along a continuous spectrum, largely depending on the host immune reaction to the pathogen. Both leprosy bacilli primarily infect and grow in macrophages and Schwann cells, with M. lepromatosis having a higher affinity to internal organs than M. leprae.

Histological descriptions

Histological descriptions of leprosy in human beings are often focused on the skin, but lesions in other tissues are essentially similar (Ridley and Jopling 1966). Leprous granulomas are rarely found in the superficial dermis only; dermis and sometimes subcutis are often involved. The presence of acid-fast bacilli (AFB) inside a nerve is diagnostic of leprosy. Criteria correlated with the histological progress and classification of leprosy lesions are: “foam cells, large globi, epithelioid cells, Langhans giant cells, lymphocytes, plasma cells, fibroblasts, a clear subepidermal zone, the cellular cuffing of nerves, and infiltration of nerves”. The five main categories to which human leprosy cases are assigned reflect a range from strong (tuberculoid, TT) to weak host immune response (lepromatous, LL), with three categories in between: borderline tuberculoid (BT), borderline borderline (BB), and borderline lepromatous (BL). Early lesions that already show some typical characteristics but do not yet fall within one of the categories are called indeterminate. The bacterial index (BIn) was introduced to comparably quantify AFB seen in skin smear samples from human leprosy patients or in histopathologic sections of granulomas. The criteria for each category are summarised in Table 1.
Table 1: Histological features of the five main forms of human leprosy (Ridley and Jopling, 1966; Massone, Belachew and Schettini, 2015)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TT</th>
<th>BT</th>
<th>BB</th>
<th>BL</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent of infiltrate</td>
<td>Dermis to epidermis</td>
<td>Dermis, seldom reaching epidermis</td>
<td>Spares epidermis, but epidermis can be atrophic</td>
<td>Spares epidermis, formation of a small band of collagen (Unna band)</td>
<td>Clearly separated from epidermis (Unna band), can reach deep into dermis</td>
</tr>
<tr>
<td>Cells observed</td>
<td>Epithelioid cells, Langhans giant cells, dense lymphocyte infiltration</td>
<td>Epithelioid cells, Langhans giant cells, dense lymphocyte infiltration</td>
<td>Epithelioid cells, some lymphocytes diffusely spread</td>
<td>Histiocytic cells, no foamy cells and scanty lymphocytes OR histiocytes with foamy changes, dense areas of lymphocyte infiltrate</td>
<td>Histiocytes with fatty changes, foam cells, multinucleate globe, few lymphocytes</td>
</tr>
<tr>
<td>Nerve involvement</td>
<td>Nerve bundles unrecognisable within granuloma</td>
<td>Swollen, infiltrated</td>
<td>Moderate Schwann cell proliferation</td>
<td>Perineural lymphocyte cuffs, loss of nerve bundle structure</td>
<td>Some structural damage, no infiltration</td>
</tr>
<tr>
<td>BIn</td>
<td>0</td>
<td>0-2 granuloma, 1-3 nerve bundles</td>
<td>3-4</td>
<td>5</td>
<td>5-6</td>
</tr>
<tr>
<td>PCR from lesion</td>
<td>Often negative</td>
<td>Positive in 50% of cases</td>
<td>Positive in most cases</td>
<td>Almost always positive</td>
<td>Always positive</td>
</tr>
<tr>
<td>Dominant Th type</td>
<td>CD4+</td>
<td>CD8+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Leprosy in animals**

It was during the search for a suitable animal model in the 20th century that natural infection with *M. leprae* in a non-human animal was first described. In the 1970’s, wild nine-banded armadillos trapped in Louisiana, USA and then housed in a specialist facility for leprosy research purposes were found to carry *M. leprae* prior to the start of the infection experiments. Clinical disease is occasionally described, usually in adult armadillos and appears as enlarged lymph nodes, non-specific abrasions and nodule-like lesions around eyes, nose and feet. Endemic infection does not appear to put wild populations at a disadvantage. Histologically, leprosy lesions in
experimentally infected nine-banded armadillos closely resemble the spectrum found in humans. Similar clinical signs have been shown to be caused by *M. leprae* in wild six-banded armadillos (*Euphratus sexcinctus*). Other armadillo species, such as Northern long-nosed armadillos (*Dasypus sabanicola*), Southern long-nosed armadillos (*Dasypus hybridus*) have been found to be susceptible to experimental infection with *M. leprae*, but the bacterium has not been isolated from wild caught individuals of these species to date. While clinical signs of leprosy have not been reported in free ranging primates, wild-caught non-human primates have developed clinical signs in captivity without experimental infection, after being in contact with leprous human beings. Affected individuals belonged to several species (white-handed gibbons (*Hylobates lar*) in Malaysia, chimpanzees (*Pan troglodytes*) in West Africa, cynomolgus macaque (*Macaca fascicularis*) in The Philippines and sooty mangabey monkeys (*Cercocebus atys*) in West Africa. Recently, *M. leprae* DNA was isolated from nasal swabs from one captive margay (*Leopardus wiedii*), one wild and one captive lowland tapir (*Tapirus terrestris*), two wild capuchin monkeys (*Sapius apella*) and one wild owl monkey (*Aotus trivirgatus*) in Brazil, none of which showed clinical signs of disease.

**Leprosy in Red Squirrels**

*M. lepromatosis* was not described in an animal host until 2014, when it was detected in Eurasian red squirrels (*Sciurus vulgaris*) in the British Isles (Meredith et al., 2014). Later *M. leprae* was detected in this species as well and it was established that infection with and without clinical signs can be caused by both bacilli (Avanzi et al., 2016). Overall, the genetic evidence available from samples collected across this range of species suggests that humans are the main host for leprosy and that other species most likely became infected from a human source. However, at least within nine-banded armadillo and Eurasian red squirrel populations, infection has since been sustained without continued re-introduction from a human source (Schuenemann et al., 2018). The Eurasian red squirrel (ERS) is a particularly fascinating leprosy host, not just because it is the only currently known non-human animal host for both leprosy bacilli, but also because the pathogen has been sustained in its populations after it was eradicated from the human population in the British Isles. Further interest comes from a conservation perspective. Eurasian red squirrels are locally endangered in the British Isles and disease threats such as squirrelpox significantly contributed to their decline. Thus, it is pivotal to understand the impact any newly discovered pathogen may have on the local populations.

Initially, *M. lepromatosis* was identified in six Scottish red squirrels, submitted for post mortem examination between 2006 and 2012 as part of a routine passive surveillance scheme. All had bilateral bulbous, hairless skin lesions on pinnae, face and hind legs (Meredith et al., 2014). Further research found *M. lepromatosis* in symptomatic and asymptomatic red squirrels in populations throughout the British Isles and identified *M. leprae* in red squirrels in three populations (Brownsea Island, Isle of Arran, Anglesey), with clinical cases occurring in one of these (Brownsea). Initial screenings for *M. leprae* and *M. lepromatosis* in Eurasian red squirrel populations in other countries such as France, Belgium, the Netherlands, Germany, Switzerland and Italy did not detect leprosy bacilli (Schilling et al., 2019). Based on initial clinical and histological descriptions of squirrel leprosy, several differential diagnoses need to be considered when assessing a clinical case (Table 2).
### Table 2: Differential diagnoses for squirrel leprosy (other diseases causing skin lesions in Eurasian red squirrels)

<table>
<thead>
<tr>
<th>Diagnosis (causative agent)</th>
<th>Clinical lesion description</th>
<th>Histological lesion description</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical histiocytosis (unknown)</td>
<td>Cutaneous, subcutaneous and/or internal swellings and nodules</td>
<td>Sheets of atypical round cells and multinucleated giant cells</td>
<td>(Smith et al, 2017)</td>
</tr>
<tr>
<td>Malignant melanoma (non-infectious)</td>
<td>Masses on eyelids</td>
<td>Highly pleomorphic epithelioid cells, some with melanin granules</td>
<td>(Fukui et al, 2002)</td>
</tr>
<tr>
<td>Poxvirus infection (Squirrelpox virus, SQPV)</td>
<td>Erythematous scabs on periorbital skin and feet, skin ulceration</td>
<td>Mixed inflammatory cell dermatitis, ulceration, ballooning degeneration of epidermal cells</td>
<td>(McInnes et al, 2009)</td>
</tr>
<tr>
<td>Poxvirus infection (Berlin squirrelpox virus)</td>
<td>Exudative and erosive dermatitis with serocellular crusts at auricles, noses and digits, tail and perineum</td>
<td>Ballooning degeneration of epidermal keratinocytes, intracytoplasmatic inclusion bodies, epidermal ulceration, secondary bacterial infection</td>
<td>(Wibbelt et al, 2017)</td>
</tr>
<tr>
<td>Fatal exudative dermatitis (<em>Staphylococcus aureus</em>)</td>
<td>Exudative scabby lesions around mouth, nose and eyelids, inflammation and sloughing of foot skin</td>
<td>Exudative, ulcerative, necrotic dermatitis with epidermal hyperplasia and hyperkeratosis</td>
<td>(Simpson et al, 2013; Blackett et al, 2018)</td>
</tr>
<tr>
<td>Abscess (for example <em>Yersinia enterocolitica</em>)</td>
<td>Facial abscesses</td>
<td>Dermal necrosis</td>
<td>(Simpson et al, 2013)</td>
</tr>
<tr>
<td>Dermatophilosis (<em>Dermatophilus congolensis</em>)</td>
<td>Raised, firm, crusty lesions with pale subcutaneous necrotic tissue</td>
<td>Orthokeratotic and parakeratotic hyperkeratosis, large numbers of viable, degenerated and lytic neutrophils embedded in cell debris and fibrin.</td>
<td>(Holmes et al, 2019)</td>
</tr>
</tbody>
</table>

Histologically, leprosy lesions described in Eurasian red squirrels show a less broad spectrum than described for humans and nine-banded armadillos, for the most part containing high numbers of bacteria and showing characteristics of BL and LL lesions. To further evaluate the question whether the whole spectrum of leprosy presentations occurs in ERS, the author’s research group collected up to 13 tissues (ear, eye, hind footpad, liver, hock skin, nose, lung, muzzle, mandibular lymph nodes, intestines, kidney, front footpad, spleen) from 11 ERS carcasses from a leprosy endemic areas; seven of these individuals showed skin lesions that were classed as signs of leprosy. In this study, it was possible to describe inflammatory reactions showing the characteristics of two additional histological categories of leprosy in ERS (InL and BB) in addition to the ones previously published (LL, BL and BT) (Avanzi et al, 2016). All
lesions seen in this study were InL, BB, BL or LL, thus further strengthening the impression that lesions on the lepromatous end of the spectrum are much more likely to be detected in ERS than tuberculoid lesions, with TT lesions remaining undescribed. The prominent nerve invasions with large numbers of AFB described for other hosts has not been observed in ERS yet, while some nerve involvement, particularly in the form of lymphocyte cuffs or individual AFB in nerves, have been seen. Within clinically visible granulomas in ERS, normal structures like nerve bundles or larger vessels were no longer identifiable. Granulomas in ERS appeared to form close to the cartilage initially and then expanded through the dermis until they reached the epidermis and, in some instances, ulcerated. This pattern is very different from what is described for BL-LL lesions in humans and is in some respects similar to what is described for TT lesions in human beings. However, other characteristics, like globi formation and large numbers of foamy macrophages, have placed ERS leprosy lesions at the lepromatous end of the histological spectrum.

To enable further research on squirrel leprosy, diagnostic methods have been recently adapted for the use in live animals. A field friendly serological test has been validated (Schilling et al, 2019) and has been used in combination with clinical assessment and pathogen DNA extraction from small tissue samples collected under general anesthesia to establish a sound approach to live squirrel testing (currently under review for publication). One outcome of this research was the proposal to slightly adapt the definition of leprosy lesions in Eurasian red squirrels, as lesions may initially form unilaterally, and the previously described bilateral distribution is mainly observed in advanced cases. Furthermore, two main sites at which lesions initially form were observed (ear and hock), based on information from 125 live squirrels and 44 squirrel carcasses assessed in two British populations between 2016 and 2018 (data submitted for publication). The impact of clinical sign on the individuals, the time scales on which leprosy develops in Eurasian red squirrels, as well as the incidence rate in affected populations are key to establishing the effect the disease may have on these populations. Longitudinal data collected in the same two British populations mentioned before has allowed initial insights into these matters, implying a slow progression of leprosy in squirrels over months and years and with limited welfare impact for a large proportion of the disease history. This data has also recently been submitted for publication.

Overall, much remains to be learned about squirrel leprosy, but the research that has taken place since its initial discovery has resulted in a very sound basis for further research. Once all data is published, future research across the full species range can build on it to collect comparable datasets to better understand squirrel leprosy epidemiology. Summaries and images aiming to assist those working closely with Eurasian red squirrels in identifying leprosy cases have been made freely available online in English, French and German (https://www.ed.ac.uk/vet/conservation-science/conservation-medicine/projects/squirrel-leprosy). While the current data suggests that squirrel leprosy is not a major threat to Eurasian red squirrels in the UK, they may offer future research opportunities, such as observational in situ transmission studies, that may significantly contribute to understanding this ancient disease.

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CAN WE SAVE THE TASMANIAN DEVIL FROM EXTINCTION?

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Welcome to the 10th World Congress of Veterinary Dermatology
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