

# **CONCURRENT SESSION PRESENTATIONS**

# ZINC-ASSOCIATED DERMATOSES OF THE DOG

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## BACKGROUND

Zinc is an essential dietary element and is incorporated into enzymes where it plays a key role in regulating various aspects of cellular metabolism.<sup>1</sup> If zinc levels are low due to insufficient dietary intake, decreased dietary absorption, competition between hormones such as estrogen and zinc for serum protein, or an inherent defect of zinc utilization or uptake at the cellular level, a disease state will likely result.<sup>1-2</sup>

Four basic groups of zinc-associated dermatoses have been reported to date in the dog. The first (Syndrome I) may occur in any age of dog fed balanced diets and Siberian Huskies appear to be over represented as a breed.<sup>1</sup> The second (Syndrome II) has been reported in young dogs fed unbalanced diets low in zinc or high in plant protein (phytate) or calcium, which binds zinc and prevents absorption. Cereal or soy based diets have also been linked to this syndrome.<sup>2</sup> The third is lethal acrodermatitis in Bull Terriers that appears to be an inherited autosomal recessive trait that produces a lethal syndrome. These patients' serum zinc levels are low, however, they do not respond to oral or parenteral zinc supplementation.<sup>3</sup> The last group reported was a litter of Pharaoh hound puppies that were severely clinically affected, exhibited low serum zinc levels and did respond favorably to intravenous but not oral zinc supplementation.<sup>4</sup>

## PHYSICAL FINDINGS

The clinical hallmarks of zinc-associated dermatoses in the dog are crusting, alopecia and erythema with variable amounts of focal pruritus. Lesions are often on the face and lip margins and are usually symmetrical, however, asymmetry is not uncommon. Crusted and alopecic lesions have also been reported on the nasal bridge / planum, footpads, perigenital region, distal limbs and the elbows.<sup>1</sup>

## DIAGNOSIS

Skin biopsy is the gold standard for confirmation of suspected zinc-associated dermatoses.<sup>1</sup> The hallmark histologic finding is parakeratotic hyperkeratosis, oftentimes extending into the follicles. Orothokeratosis and acanthosis are also common findings and may be present concurrently with the parakeratosis. One must be certain that all skin infections (especially *Malassezia* dermatitis) are cleared prior to biopsy and that surgical scrubbing is NOT performed as these mistakes will readily lead to a misdiagnosis.

## PROGNOSIS

Syndrome I and II patients typically have good to excellent response to therapy with dietary correction (Syndrome II only) and oral zinc supplementation.<sup>1</sup> Bull Terriers with lethal acrodermatitis carry a uniformly grave prognosis<sup>5</sup> and the Pharaoh hound disease appears to have a prognosis between the other reported diseases as they did respond favorably to intravenous zinc infusions.<sup>4</sup>

## THERAPY

Lifetime supplementation of zinc will likely be required for all patients but the Syndrome II puppies with dietary deficiencies. Oral products such as zinc sulfate, zinc methionine and zinc gluconate are dosed based on the elemental zinc present at dosages between 1.2 and 5.1mg kg<sup>-1</sup>. Patients appear to have variable zinc absorption and the wide dosage range appears to reflect the need to specifically tailor dose to the patient. Improvement is typically noted within 6 weeks. Intravenous therapy with zinc infusions as previously described using 10-15mg kg<sup>-1</sup> of zinc sulfate (basing dose on elemental zinc content) then diluted 1:1 and administered IV very slowly once weekly<sup>5</sup> can lead to severe gastrointestinal symptoms of vomiting and bloody diarrhea. If attempting intravenous IV infusion of zinc, this author recommends to start with 3-5 mg kg<sup>-1</sup> diluted 1:1 with saline, administer very slowly (over several hours) then gradually increase the dose on subsequent infusions as needed to balance side effects and clinical efficacy.

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## NOTES

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# EUROPEAN CANINE LEISHMANIOSIS

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## INTRODUCTION

Canine leishmaniosis is a major infectious and zoonotic disease in many parts of the world. European canine leishmaniasis is caused by the diphasic protozoan intracellular parasite *Leishmania infantum*, transmitted by sandflies of the genus *Phlebotomus*. It is mainly a Mediterranean disease but it is currently expanding northerly. Surveys based on polymerase chain reaction (PCR) and serology show an infection rate up to 70 % in enzootic areas i.e. much higher than previously thought, with millions of dogs infected. As many companion animals travel all over Europe and beyond, cases can be seen far from the enzootic areas, in Europe but also in North America.

## AETIOLOGY, PATHOGENESIS AND EPIDEMIOLOGY<sup>1-3</sup>

The sandflies (phlebotomes) live in adapted biotopes (rocks, dry areas with humid micro-habitats, moderately high temperatures) and their activity is mainly crepuscular. Only the adult females are haematophage, but not the males. The infection of dogs by *L. infantum* depends on the phagocytosis and transformation of promastigotes (the extracellular and flagellated form in sandflies) into intracellular amastigotes, the latter being much more resistant to cellular defense mechanisms. Transmission other than via sandflies, e.g. *in utero*<sup>4</sup>, venereal<sup>5</sup> or by blood transfusion has been demonstrated and transmission by contact<sup>6</sup>, via fleas and ticks<sup>7</sup> is likely to be possible but not well documented. They probably do not play an important role.

There is a “new paradigm” of canine leishmaniosis (L Ferrer). Some contaminated dogs will develop the clinical disease but not all: some others will develop a persistent asymptomatic infection (and perhaps will develop the disease eventually) and there is a third group of dogs that are resistant to or eliminate the infection without developing clinical signs. In other words, there are “susceptible” vs “clinically resistant” dogs (due to a cellular immune response) that are seronegative or have borderline titers. A study using the polymerase chain reaction (PCR) techniques on different tissues has demonstrated that two thirds of dogs are infected in Mallorca, most of them showing no clinical signs<sup>8</sup>. Genetic factors are probably involved. For instance, Ibiza hounds, a breed originating from the Balearic Islands show a predominantly cellular response, which is protective<sup>9</sup>. In contrast, other breeds such as Boxer, Cocker spaniel, Rottweiler and German shepherd seem to be predisposed<sup>10</sup>. In addition, it has been shown that susceptible dogs in Europe have a mutation in a particular gene (Nramp1 or Slc11c1). This gene controls an ion transport protein involved in the intraphagosomal replication of amastigotes<sup>11</sup>. However, the genetics of resistance/susceptibility is probably complex and several genes are involved, as demonstrated in human leishmaniosis and in murine models.

The prevalence of the clinical disease in enzootic areas is usually below 10% and dogs that develop it have a predominant humoral response and are strongly immunodepressed biologically (poor response in lymphocyte proliferation test, low cytokine production by peripheral blood mononuclear cells after stimulation, low number of circulating CD4<sup>+</sup> cells and decreased CD4<sup>+</sup>/CD8<sup>+</sup> ratio). In contrast dogs which develop a cellular immune response (Th 1 type) are more resistant. Alterations in immunological status can trigger the disease (immunosuppressive drugs, other parasitic or infectious diseases, neoplasia). The incubation period is quite variable, extending from a few months (incubation *sensu stricto*) to several years in case of re-activation of a latent, controlled infection.

In clinically affected animals, the first stage of the disease is a transient cutaneous phase (inoculation sore, up to a few months) followed by a general dissemination of the parasite. The inflammatory process of humoral immune response (circulating immune complexes) induces lesions leading to the appearance of clinical signs from different systems, and particularly from the kidneys<sup>1,2</sup>.

Epidemiological data are available from the EU southern countries, the northern coast of the Mediterranean sea and Israel<sup>1,2,12</sup>. In France, the south-east and Corsica constitute the enzootic area but it is expanding northerly and easterly (Bourdeau et al, Canine leishmaniosis in France: A national survey on distribution and prevalence. Multicolloquium of Parasitology, Valencia, Spain, July 2004, 172). *Leishmania infantum* is mainly transmitted by *Phlebotomus perniciosus* (in sub-urban areas) or *Phlebotomus ariasi* (in the country). In man, about 15 cases are reported per year in southern France (i.e. one human case per a few hundred canine cases). A new aspect is the increasing incidence of the disease amongst adult humans (50 % of cases) due to iatrogenic immunosuppression or AIDS. In Spain, canine leishmaniosis is seen in almost all the country and in the Balearic Islands, except the northern coast, with *Phlebotomus perniciosus* as the main vector. In Portugal, the disease seems to be spread out all over the country, including the north. In Italy, canine leishmaniosis is seen in most of the country, especially in the centre (up to Bologna) and the south, as well as in Sicily and Sardinia. It is expanding towards the North<sup>13</sup>. In Greece and Israel the disease is ubiquitous. The disease is present on the African Mediterranean coast, including in northern Morocco<sup>14</sup> and Tunisia.

## CLINICAL SIGNS<sup>1,2,15-17</sup>

Very young dogs are rarely affected whereas there are two peaks of prevalence: 2 to 4 years and above 7 years of age<sup>10</sup>. Sex predisposition for male dogs has been reported, perhaps due to behaviour<sup>10</sup>, but is still controversial. Cutaneous lesions are probably the most common clinical manifestations of this multisystemic disease. These include psoriasiform scaling on the face, ear pinnae, limbs that eventually becomes generalized, erosions/ulcers particularly on the ear pinnae, bone prominences and footpads, papules and nodules (including inoculation sores), sterile pustulosis, nasal (and lip/oral) depigmentation, onychogryphosis and paronychia<sup>18</sup>, plaques (rare), oedema (face or legs, due to vasculitis, nephrotic syndrome or more rarely to blood hyperviscosity caused by hyperproteinaemia), purpura (rare), and secondary pyoderma (common). The systemic disease is characterized by prostration, progressive weight loss, lymph node enlargement and splenomegaly, chronic renal insufficiency (glomerulonephritis), splenomegaly, epistaxis, ulcerative stomatitis, haemorrhagic enteritis, neurological disorders (meningitis), polyarthritis and osteomyelitis leading to lameness or limb deformity, and osteitis of the 3<sup>rd</sup> phalanx. Ocular signs are common and include conjunctivitis (which can be nodular), keratoconjunctivitis sicca, uveitis, glaucoma and panophthalmia. Atypical forms are numerous and are confirmed by the identification of leishmaniads in samples<sup>19</sup>.

Canine leishmaniosis, with its wide range of clinical manifestations, can mimic a large number of diseases and should be suspected in case of keratoseborrhoeic disorder, chronic ulcerative skin lesions, recurrent and resistant deep pyoderma and multisystemic disease. Leishmaniosis is a real impersonator.

A staging has been proposed by the LeishVet group<sup>2</sup>. It can be briefly summarized as follows:

- Stage I: mild clinical disease e.g. lymphadenopathy or papules, no clinicopathological abnormalities, negative or low level serology
- Stage II: moderate disease defined as Stage I plus more cutaneous lesions and systemic signs, clinicopathological signs, without (Stage IIa) or with (Stage IIb) abnormal renal profile and a low to high positive serology level
- Stage III: severe disease defined as Stages I and II plus severe systemic and ocular signs, more severe abnormal renal profile and medium to high serology level
- Stage IV: very severe disease defined as Stage III plus very severe systemic signs including nephrotic syndrome and/or end stage renal insufficiency.
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This staging system is useful to both the prognosis and the selection of the most appropriate therapy.

## DIAGNOSIS<sup>2,3,12</sup>

The purposes of diagnosis include both the confirmation of the disease in dogs with clinical signs and/or clinicopathological abnormalities and the diagnosis (screening) of infection in dogs for various reasons (healthy dogs being infected or not)<sup>2,12</sup>.

History, including epidemiological data for a particular patient, is extremely important (previous or current stay in an enzootic area). Physical examination should be exhaustive since leishmaniosis is a multi-systemic disease, with a wide range of clinical signs. The diagnosis of canine leishmaniosis is often a challenge<sup>20</sup>.

Laboratory findings can be suggestive of leishmaniosis. Proteinuria is common. Haematology shows mild anaemia (normochromic, normocytic) and thrombocytopenia. Biochemistry shows hyperazotaemia, hypercreatininaemia, hypoalbuminaemia, hyperproteinaemia and an abnormal serum protein electrophoresis pattern (decrease of albumin, increase of  $\beta$  and  $\gamma$ -globulins).

Cytological demonstration of the presence of amastigotes is obviously diagnostic of infection and, if associated with lesions, is also conclusive of disease. Amastigotes can be found in cutaneous (or conjunctival) smears, lymph node aspirates, bone marrow aspirates, and spleen aspirate (which can be dangerous in dogs with thrombocytopenia). May-Grünwald-Giemsa or rapid stains are appropriate. These techniques have a poor sensitivity, even in severe cases and if negative no conclusion can be drawn. However the clinician's expertise could increase this sensitivity.

Serology is the traditional diagnostic method in both symptomatic and asymptomatic dogs. Quantitative serological assays detect anti-*Leishmania* antibodies: indirect immunofluorescence assay (IFA), enzyme-linked immunosorbent assay (ELISA) including with the purified antigen rK39, immunoprecipitation (electrosyneresis), agglutination latex (including the in practice technique), Western Blot (not a routine technique). Sensitivity and specificity are usually good (cross reactions are rare). Results can be negative, positive or ambiguous. A high titre is diagnostic but if borderline, the test should be repeated three months later. In the meantime, other diagnostic methods can and should be used.

Dermatopathology can be fruitless, suggestive or diagnostic. In the keratoseborrheic, ulcerative and nodular cutaneous forms, a nodular to diffuse granulomatous dermatitis, perifolliculitis and/or sebaceous adenitis can be seen, with variable numbers of lymphocytes, plasma cells and neutrophils. Numerous amastigotes are usually found in the nodular form, but they appear in variable number in the keratoseborrheic form and are rare in the ulcerative form (as a mean they are found in 50 % of the cases and their number decreases with chronicity). In the sterile pustular form the pustules are subcorneal or intraepidermal, neutrophilic and acantholytic, with few amastigotes in neutrophils and dermal macrophages. More rarely interface lichenoid dermatitis or nodular dermatofibrosis are found. Cutaneous leishmaniasis should be differentiated from mycobacterial infections and sterile granulomas/pyogranulomas<sup>21</sup>. Immunohistochemical staining (e.g. immunoperoxidase) and PCR are useful. It should also be differentiated from idiopathic sebaceous adenitis which shows no dermal inflammation<sup>22</sup>. Histopathological examination of other organs can demonstrate the presence of lymphohistiocytic or granulomatous inflammation along with a variable number of amastigotes (eventually with the aid of immunoperoxidase staining).

Polymerase chain reaction (PCR) using genomic or kinetoplast DNA, performed with skin, lymph node, bone marrow, spleen and biological liquids (aqueous humour, synovia, cephalorachidian liquid, bronchoalveolar lavage fluid), can be useful in demonstrating that the sample and, in consequence, the animal harbour leishmaniads (kDNA assays are the most sensitive). Quantitative real-time PCR can quantify the number of parasites in the sample and also detect very low parasitic loads. It is important to remember that PCR means only that the parasite has been inoculated (it does not mean that there is infection i.e. that the parasite is alive and in a multiplication phase) and its result should be correlated with the clinical signs and above all serology. Clinically healthy dogs and dogs with other diseases than leishmaniosis may be PCR positive and treating a dog exclusively on the basis of a positive PCR is not justified.

In summary the final diagnosis of canine leishmaniosis is a combination of compatible epidemiological data, clinical signs and several diagnostic tests, of which the most important is quantitative serology.

In canine dermatology, the differential diagnosis of leishmaniosis includes autoimmune and immune-mediated dermatoses (pemphigus foliaceus, sub-epidermal bullous diseases, cutaneous and systemic lupus erythematosus), granulomatous sebaceous adenitis, demodicosis, dermatophytosis, pyoderma, all keratoseborrheic and ulcerative disorders, nodular neoplasia and pseudoneoplasia.

## **THERAPY**<sup>2,12,23</sup>

The classical drugs used to treat canine leishmaniosis are N-methylglucamine antimoniate (Glucantime®, Merial) and allopurinol. Glucantime® inhibits ATP synthesis in glycolytic oxidation and EFA metabolism. The Merial company recommended at the time of licensing a dose of 200 to 300 mg/kg of Glucantime® subcutaneously every 2 to 3 days with a total of 20 injections, but there is a consensus and good evidence to recommend the daily dose of 100mg/kg for 21 to 28 days, subcutaneously<sup>21</sup>. It has been shown that IV and SQ routes are equivalent<sup>24</sup> but the IV route allows a quick elimination and is perhaps not recommendable. Allopurinol is used for the treatment of hyperuricemia in man but it has also the characteristic of inhibiting the growth of kinetoplastidae by means of a metabolite incorporated in the RNA of the parasite with a lethal effect. Allopurinol is administered *per os* at the dose of 10 mg/kg BID. In fact the combination of both products is recommended and allows a long term survival: Glucantime® for up to 30 days and allopurinol indefinitely or until full clinical recovery is achieved and antibody titre is very low (close to or at the cut-off level)<sup>25,26</sup>, which takes months or years to achieve. Rarely, immune-mediated lesions and urolithiasis can occur<sup>26</sup> but they are reversible after discontinuation of therapy. Allopurinol monotherapy (10 mg/kg TID) has been proposed in non-enzootic areas where inoculation is not continuous<sup>27</sup>. Miltefosine (Miltefosan®), a phospholipid of high leishmanicidal activity, has been demonstrated recently to be as effective as Glucantime® particularly in association with allopurinol as well<sup>28</sup>. It is a good alternative to Glucantime® at the dose 2 to 3 mg/kg/day. Side effects are gastrointestinal and the product is highly teratogenic including after discontinuation of therapy. Amphotericin B (Fungizone®) has been used at the dose of 0.5-0.8 mg/kg 2 to 3 times per week in rapid IV (30-45 seconds) with a checking of creatinine before each injection (treatment is halted if > 25 mg/l)<sup>29</sup> and also in lipid emulsion with good results<sup>30</sup>. Nephrotoxicity is the main concern of its use. New formulations of amphotericin B (Liposome: Ambisome® or lipidic complex: Abelcet®) are also effective and less toxic<sup>31</sup> but are very expensive. Pentamidine (Lomidine®), allylamines and azole derivatives are toxic and not effective, respectively, thus not used anymore. Marbofloxacin (2mg/kg SID for 28 days) improves the clinical status of some affected dogs<sup>32</sup> but the use of a fluoroquinolone for treating canine leishmaniosis is questionable. Amminosidine sulphate or paromomycin, is an injectable aminoglycoside antibiotic (Amminofarma®) available in Italy and Greece. It is given at the dose of 15 mg/kg SID but has serious side effects (nephrotoxicity and neurotoxicity). Recently, domperidone, a dopamine D2 receptor antagonist, has given interesting results in a study, at the dose of 1mg/kg BID for 1 month<sup>33</sup>.

Some concerns have been expressed about the use of miltefosin and amphotericin B in dogs because these drugs are used to treat humans (the former in developing countries and the latter in Europe) and their use could increase the risk of resistances.

Probably the definitive elimination of the parasites is not possible and the main scope of the treatment is to help the immune system to control the infection and to achieve a clinical improvement which can be temporary. Therefore, follow-up is extremely important. Since serology titres decrease slowly (in several months or years), usually (but not always) along with clinical improvement, regular monitoring is essential<sup>2</sup>. A good prognosis can be given if the serology titre and/or beta 3-gamma fractions of protein electrophoresis decrease. A relapse is likely to occur if they increase. This may indicate the need to use another antileishmanial drug. In addition, haematology and clinical biochemistry should be monitored in each case, as regularly as possible, particularly to evaluate the renal function. Regular re-checks are recommended (every 1 to 3 months at the beginning, and every 6 months on a long-term basis). There is a certain percentage of failures if monitoring is not adequate and euthanasia may be considered by the owners with whom communication and cooperation are essential.

Twenty years ago, it was considered that all seropositive animals should be treated, even if asymptomatic since therapy (Glucantime®) delays the onset of clinical signs which are likely to occur in dogs showing a humoral



immune response and the efficacy of Glucantime® could decrease with time<sup>34</sup>. The LeishVet group has proposed a new approach according to the stages<sup>2</sup>:

- Stage I: Scientific neglect is conceivable, or one course of treatment (Glucantime® or Miltefosan®, with or without allopurinol). Prognosis is good.
- Stage II: Treatment with Glucantime® + allopurinol or Miltefosan® + allopurinol is recommended. Prognosis is good to guarded<sup>26</sup>.
- Stage III: The same treatment as for stage II is recommended plus a careful monitoring of renal function (adherence to the International Renal Interest Society – IRIS guidelines for chronic kidney disease – CKD). Prognosis is guarded to poor.
- Stage IV: The recommended treatment is allopurinol alone and adherence to the IRIS guidelines for CKD. Prognosis is poor.

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Glucocorticoids (prednisolone 1 to 2 mg/kg/d) are useful in case of uveitis. Other symptomatic treatments can also be useful (e.g. non-steroidal anti-inflammatory agents, antiseborrhoeic topicals).

## PREVENTION

Sanitary prevention by euthanasia of affected individual dogs is not necessary since human and canine direct contamination is extremely rare. The reservoir is composed of dogs but also other *canidae* (e.g. foxes) and rodents. Massive canine euthanasia of seropositive dogs has failed in enzootic area in other parts of the world and is ethically unacceptable (!).

Medical prevention can be empirical. A treatment every 5 to 6 months with Glucantime® could perhaps prevent the relapses but this is not recommended (toxicity, resistance). A life-long chemioprevention with allopurinol alone may be considered. A “scientific” prevention can be achieved by routine serological examinations (at least every 6 to 12 months).

Vaccination<sup>2,12</sup> against canine leishmaniosis is a real challenge, because of the complexity of the immunomechanisms involved in the disease. Adjuvants play a major role. Four classes of vaccines have been evaluated in dogs. Purified *Leishmania* fraction vaccines are the most promising. One of them (“FML-based” Leishmune®, Fort Dodge) has been licensed in Brazil. Another one (containing the “LiESAp” antigen) produced in France by Virbac with muramyl peptide as adjuvant (CaniLeish®) has shown a high efficacy rate in a double blinded field trial<sup>35</sup>. It has been recently approved by the European Medicines Agency (EMA). Recombinant and DNA vaccines seem to be less effective.

The use of insecticides has been recommended to prevent the bites of phlebotomine sandflies<sup>2,12</sup>. The reduction of the number of phlebotomine sandflies that succeed in feeding is the summation of several possible contributory factors, including repellency and/or flushing effects and non-lethal and/or lethal knock-down activity. This is the “prevention of bite effect” exhibited by some synthetic pyrethroids. Few comparative data have been published on anti-feeding effects of insecticides against phlebotomine sandflies. A deltamethrin collar, pyrethroid spot-ons and sprays, a permethrin and pyriproxifen spray, a permethrin and imidacloprid spot-on, have been shown to be potentially effective in the protection of dogs from bites of phlebotomine sandflies, and field studies have shown that such measures reduce disease transmission and incidence in dogs, i.e. are protective. In Iran, the massive use of insecticide-impregnated dog collars reduced the incidence of leishmaniosis in dogs but also in children: the effect for the community is well demonstrated<sup>36</sup>. In addition the use of such compounds would prevent the extension of leishmaniosis, because phlebotomine sandflies can be killed by the medication either before or after having taken a blood meal, thus preventing them from searching for another host. Travellers to the endemic areas should adopt preventative measures to decrease the risk of transmission to their dogs: keeping the dogs indoors from dusk to dawn and by applying appropriate insecticides during the sand fly season (April to October in Europe).

## CONCLUSION

Recent studies have improved our knowledge on canine leishmaniosis, which can evolve into a severe disease although far from all infected dogs will develop clinical signs and/or clinicopathological abnormalities. Canine leishmaniosis is now considered an infectious disease with a strong immunological and genetic background, influencing both the diagnostic and therapeutic approach. Therapy and control can be achieved in many cases but careful monitoring is essential. Preventative measures include the use of appropriate insecticides and subunit vaccines with promising results. Leishmaniosis is a zoonosis and the veterinarian has a responsibility in the management of this disease, as a public health mission.

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## NOTES

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# HERPES AND OTHER VIRAL DERMATOSES

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When the conference organizers asked me to talk about viral dermatoses in cats, I could think of three that I had treated over the years, and only one that I considered myself to have some expertise in. So, the first part of this talk will focus on a “Cook’s tour” of viral skin disease in the cat (borrowing some slides from colleagues overseas and Wikipedia), followed by a more in-depth account of the one condition where I consider myself to have some expertise. I will also inject some history and patriotism, because I am an Aussie (Australian), and I cannot help it! The second part of the talk will describe how new therapy for FHV-1-associated dermatitis has evolved over the last few years, and potential future directions concerning anti-viral therapy in cats.

First, let’s come up with a list of viruses that have been associated with cutaneous lesions in cats:

- ✚ **Feline leukaemia virus (FeLV)**
- ✚ **Feline Immunodeficiency virus (FIV)**
- ✚ **Feline sarcoma virus**
- ✚ **Feline papilloma virus (Bowens-like disease)**
- ✚ **Pox**
- ✚ **Feline calicivirus**
- ✚ **Feline herpesvirus-type 1 (FHV-1)**

**FeLV:** A large number of skin conditions have been associated with FeLV viz. recurrent abscesses, cellulitis, paronychia, neoplasia, severe pruritus and poor wound healing. Most of these entities have implicated underlying immunodeficiency as the causal link between FeLV and these conditions. Because FeLV infection is less prevalent, not much has been written in relation to the potential association, but perhaps the clinician should consider FeLV testing in situations in which disease seems unusual, inexplicable or refractory to standard therapy, or recurs despite appropriate therapy.

**FIV:** Cutaneous conditions associate with FIV include chronic otitis externa, Demodecosis, gingivostomatitis and multiple squamous cell carcinoma of the digits, although the latter may actually have been manifestation of the lung digit syndrome. As for FeLV, most of these tentative associations are said to be secondary to secondary immune suppression.

**Feline sarcoma virus:** This is a recombinant version of FeLV that causes multiple cutaneous fibrosarcomas in cats less than 3-years of age. I have not seen this, presumably because it is rare in places like Australia where FeLV is comparatively rare.

**Feline papilloma virus:** This virus causes papillomas in middle-aged to older cats. Tumours tend to be situated on the head, eyelids and feet. They are usually solitary, small and well circumscribed, and may be pedunculated or cauliflower like. These lesions can generally be treated by simple surgical excision. Occasionally they ulcerate and bleed. Feline papilloma virus also causes multiple squamous cell carcinomas *in situ*, also known as Bowen’s like disease. This is an emerging entity in cat medicine, in which plaque-like papillomatous lesions develop in multiple locations over the cats. To my mind, the lesions are somewhat reminiscent of dermatophilosis in horses. Bowenoid dermatopathy in cats is usually quite characteristic, and a presumptive diagnosis can generally be made from the characteristic appearance and distribution of the lesions, although a definitive diagnosis is usually obtained using biopsy, histology (viral inclusion bodies), immunohistology (for papilloma virus antigen) or PCR (for papilloma nucleic acid). Some years ago, it was thought this disease was usually a marker for underlying immunodeficiency, and that affected cats would generally develop a sinister disease (usually neoplastic) within a

year of being diagnosed, however the more recent literature does not bear this out. The lesions can be treated in a variety of ways – systemic interferon, intralesional interferon, topical Aldara, cryosurgery (using a liquid nitrogen applicator), simple surgical excision, or beta irradiation from a strontium-90 applicator.

**Pox:** The pox is related to the vaccinia virus, and got its name from the distribution of the disease when dairymaids touched the udders of infected cows. The ailment manifests itself in the form of red blisters and is transmitted by touch from infected animals to humans. Cowpox is similar to but much milder than the highly contagious and sometimes deadly smallpox disease. The virus is found in Europe, and mainly in the UK. Human cases today are very rare and most often contracted from domestic cats. The virus is not commonly found in cows; **the reservoir hosts for the virus are woodland rodents, particularly voles.** It is from these rodents that domestic cats contract the virus. Symptoms in cats include lesions on the face, neck, forelimbs, and paws, and less commonly upper respiratory tract infection. Lesions may be pruritic and are variable in character, including erythematous macules, papules and plaques, crusts over purulent ulcers and ulcerated nodules. Initial lesions are usually solitary and often located on the head, neck or forelimbs, but become generalised. Buccal ulceration and systemic signs (fever, dyspnoea, and diarrhoea) may also occur. Most cats recover spontaneously. Diagnosis is easily obtained by examination of cutaneous lesions histologically and using immunohistology and PCR. Symptoms of infection with cowpox virus in humans are localized, pustular lesions generally found on the hands and limited to the site of introduction. The incubation period is nine to ten days. The virus is prevalent in late summer and autumn.

**Feline Calicivirus:** This epitheliotropic viral disease was first described as a cause of skin disease in 1970 by Margaret Sabine, an Australian veterinary virologist who died earlier this year. The paper is not well known because it was published in the Australian Veterinary Journal (in 1972) – and rather cheekily, Cooper and Sabine called the condition “paw and mouth disease”. The lesions in affected cats consisted of vesicles, erosions and ulcers located on the feet and oral cavity of young cats. It was postulated that the ulcers were the result of virus from oral cavity being inoculated into susceptible skin by licking, which proved sufficient micro trauma and maceration to facilitate viral entry into the epidermis. Lesions healed spontaneously, or with symptomatic and supportive therapy. Another Calicivirus syndrome was described by Sabine’s PhD student and later colleague, Daria Love, who described (with Max Zuber) a syndrome of severe febrile systemic illness that culminated with the appearance of perianal ulceration. Cats improved spontaneously shortly after the appearance of ulcers in the peri-anal skin.



**Figure 1. “Paw and mouth disease” in a Burmese kitten due to transference of Calicivirus from oral cavity to dorsal forelimb, by licking.**

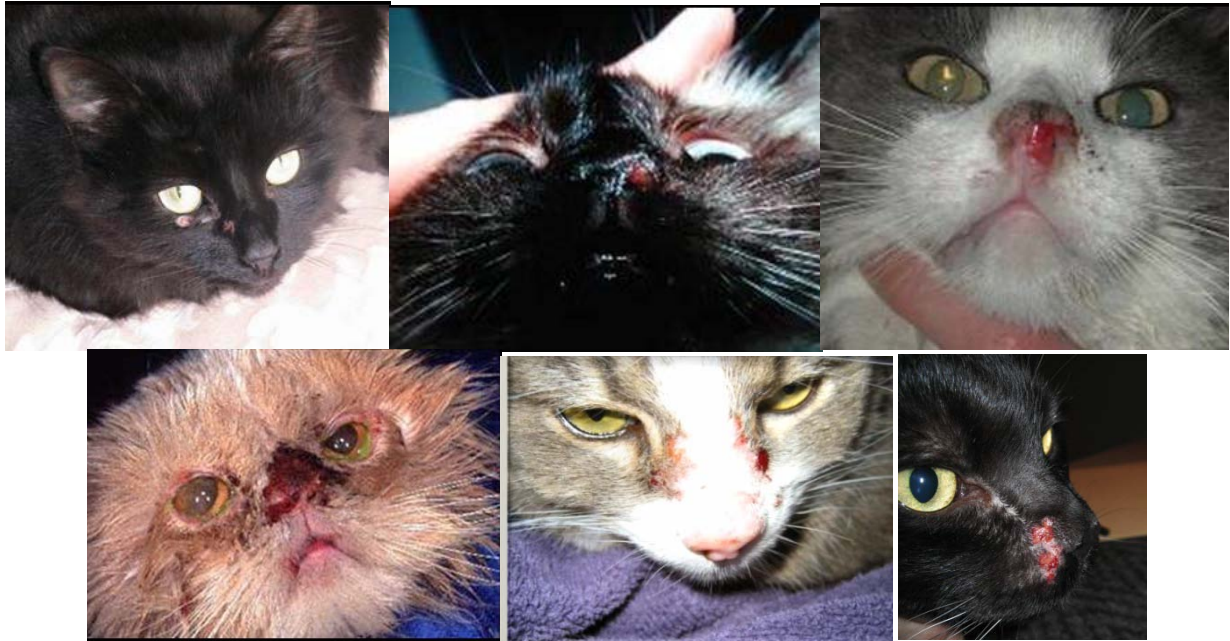
**FHV-1:** This  $\alpha$ -Herpes virus was first shown to be associated with cutaneous disease by Margaret Sabine and Roger Johnson in 1971. This seminal paper described cutaneous ulcers on the thorax, abdomen and limbs of cats. A subsequently paper in 1979 by a British group described similar findings. For some reason the more common syndromic presentation of FHV-associated dermatitis was not evident in these early papers, and the entity remained poorly appreciated till some twenty years later (see below). Interestingly, erosive changes at the medial

canthi and around the nares were seen historically in the UK in cats administered combined intranasal calicivirus and FHV-1 vaccines, although it is not clear which of the live attenuated viruses were responsible for the lesions.

## A STORY OF EVOLUTION IN FELINE THERAPEUTICS

### THE BEGINNING

In 2002, Helen Powers was invited to speak at the *Australian College of Veterinary Scientist's "Science Week"*. Helen had a novel career path. She completed an internship and residency in large animal medicine at Cornell University. After time in both equine and small animal practice, she had an epiphany and retrained in dermatology at UC Davis. Helen currently runs a busy dermatology referral service in California.



**Figure 1.** Montage of cats with FHV-1 associated dermatitis. Note that lesions can be of variable severity, extent and depth of involvement, and are located on different parts of the cat's face, although they are generally situated in the vicinity of the nares or eyes.

Helen presented a number of talks to the **Dermatology Chapter** of the College, but the one relevant here was her description of the dermatitis associated with feline herpesvirus-type 1 (FHV-1). There were two reasons why her talk got our attention. First, the audience by and large, was not aware of the entity, despite it having been first described in Australia by Margaret Sabine in 1970. Helen described a clinical picture that was syndromic: **lesions typically consisted of inflamed and often ulcerated regions in the vicinity of the nares (Figure 1). These lesions were not pruritic or painful, and failed to respond to antibacterial therapy. Usually there is a recent history of an upper respiratory tract (URT) infection in the weeks prior to the development of lesions; nasal signs typically resolve, however cutaneous lesions persist.** In the 1970s the diagnosis relied on detecting viral inclusion bodies in the biopsy specimens and/or isolating FHV-1 in cell culture. Because viral inclusions are hard or impossible to find and tissue culture was never routinely available to practitioners in private practice, a definitive diagnosis was not easy to make. Furthermore, therapeutic trials were not possible as appropriate drugs were not then available.

Around 1999, two key papers redefined the syndrome and established **the prominent role of eosinophilic inflammation within the lesions.** Either the polymerase chain reaction (PCR) or immunohistology were utilised to detect viral nucleic acid or FHV-1 antigens, respectively, in cases that fitted the clinical picture but in which inclusion bodies were absent. This work has subsequently been repeated and expanded by several other groups.



Nowadays, detecting FHV-1 antigen in affected tissues using immunohistology is proving to be the easiest way to confirm a presumptive clinical diagnosis in private practice. **A fascinating feature of FHV-1-associated dermatitis is the prominence of eosinophils in the inflammatory infiltrate, which prior to the development of PCR and immunohistology, often led to an erroneous diagnosis of eosinophilic granuloma complex and inappropriate therapy with corticosteroids, megestrol or other immunomodulatory agents, often with most unsatisfactory results. Another important differential diagnosis for FHV-associated dermatitis is insect-bite hypersensitivity.** Cheetahs can develop FHV-1 related dermatitis sufficiently severe to be life-threatening.

Helen went onto introduce audience to a new drug – famciclovir – which she said was proving very useful in the management of FHV-1 infections in cats, including FHV-1-associated dermatitis. Graciously, Helen credited the initial use of this drug to veterinary ophthalmologists in private practice in California, and her colleague Carlo Vitale, originally from Erie (Pennsylvania), who also trained with the dermatology group at Davis. We were excited that there was a drug that could be used to treat FHV-1, because up until then acyclovir, the drug that had revolutionized treatment of systemic and local alpha-herpesvirus infections in people could not be used in cats because of the risk of life-threatening hepatotoxicity, while L-lysine was only modestly effective at treating active disease due to FHV-1. Subsequent key word searches through CAB and Medline using the terms **famciclovir** and **cat** didn't reveal any papers in the peer-reviewed literature. Interestingly, famciclovir was often mentioned on internet list serves especially amongst veterinary ophthalmologists. So we made a mental note to trial this drug in presumptive FHV-1 cases where conventional therapy (L-lysine, idoxuridine eye drops etc.) had failed or was not practical.

### **TOPICAL ACICLOVIR THERAPY**

The first potential case was a cat with FHV-1 associated dermatitis seen by Miriam Meek and Paul Gotis Graham at Rose Bay Veterinary Hospital. The patient presented for non-healing ulcers on the nasal bridge associated with eosinophilic inflammation but without viral inclusion bodies. We were all keen to trial this cat on Famvir®, but while we were waiting for the drug to be delivered to the local pharmacist, Mim started the cat on a topical human cold sore cream (acyclovir 5% w/v; Zolaten®), and the cat ulcers got better before we could try the much more expensive Famvir!

### **PRELIMINARY EXPERIENCES WITH FAMCICLOVIR**

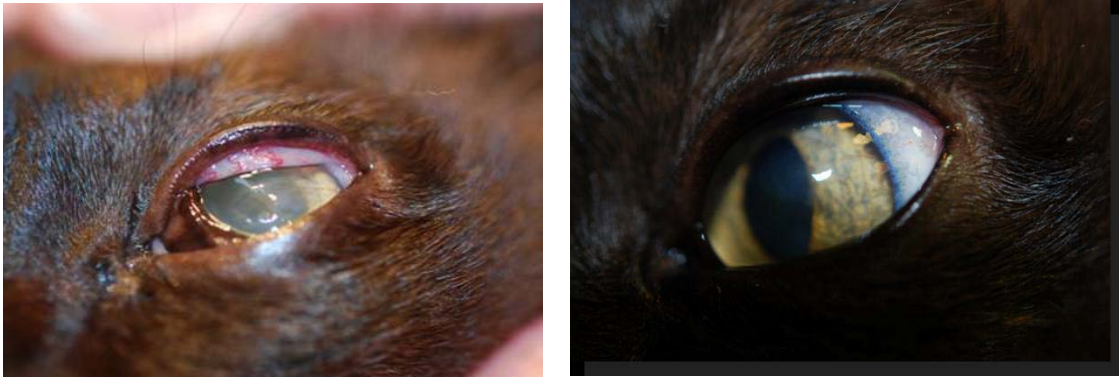
Several months later, we got another chance to trial famciclovir. A Devon Rex cat with urticaria pigmentosa being treated with cyclosporine developed recurrent bouts of FHV-1 associated conjunctivitis and ulcerative keratitis. This led to the development of a small corneal sequestrum. We e-mailed Helen and got “her” dose for Famvir® and treated the cat. Its keratitis and conjunctivitis improved markedly, the amount of brown material around the eye's decreased, and after a few weeks, the corneal sequestrum fell off.

Over the next three years, we had the opportunity to trial famciclovir in a variety of cats with different types of FHV-1 infection. Cats with various skin and ophthalmic manifestations were treated, including cases with recurrent conjunctivitis (non-responsive to doxycycline systemically and chloramphenicol topically), ulcerative keratitis (with dendritic or geographical ulcers) and corneal sequestra. Dr Sarah Webb trialled famciclovir in a cat with severe destructive rhinosinusitis. Glyn Boobyer used the drug extensively in “rescue kittens” with nasocular disease (“cat flu”). Ildiko Plaganyi used famciclovir in kittens with viral URT disease at the Lort Smith Hospital/Shelter in Melbourne. Aine Seavers (Oak Flats Veterinary Hospital) experimented with using higher doses given for shorter periods in cats with recurrent Herpesvirus ketatoconjunctis. Sally Ann Williams (Auchenflower Veterinary Hospital) (Figure 2) and Anne Fawcett and Angela Phillips (Sydney Animal Hospitals, Inner West) treated additional cases with ulcerative keratitis and/or corneal sequestra, and Mandy Burrows (Murdoch University) alerted several colleagues in WA of the value of Famvir for FHV-1 related dermatitis. All these studies were greatly facilitated by the donation of a substantial amount of free product by the human division of Novartis, the company that developed this drug.

Our preliminary experiences with famciclovir could be best described as empiric and anecdotal. When we started treating patients, pharmacokinetic data was lacking, and we extrapolated dose rates from human medicine, and the



experiences of Helen Powers and Carlo Vitale. Helen was conservative, and started with 62.5 mg per cat once day, increasing to 62.5 mg twice daily (for an average cat); this was the dose which we used most commonly for a couple of years. Carlo routinely used twice this dose in the cases he treated in his busy practice. In order to encourage other practitioners to start using Famvir®, we wrote up the first series of cases and submitted the manuscript to the **Journal of Feline Medicine and Surgery**. We were fortunate that both the reviewers and the editor (Andy Sparkes) were generous and flexible enough to see the usefulness of the work, despite its limitations.



**Figure 2. Photographs of a Burmese cat with FHV-1 associated keratitis and conjunctivitis before and after oral famciclovir therapy, and adjunctive topical therapy with hyaluronic acid eye drops.**

#### **DEFINITIVE WORK BY THE DAVIS GROUP**

Around the time we were collating our case material for publication, the first paper by ex-patriot Australian David Maggs group from UC Davis on the pharmacokinetics of famciclovir and its primary (active) metabolite penciclovir appeared in the **American Journal of Veterinary Research**. It turned out that the pharmacokinetics of famciclovir in the cat were complex and non-linear. Doses of 90 mg/kg every 8 hours produced optimal blood levels that inhibited (*in vitro*) the type strains of FHV-1 used by Maggs and colleagues. Such high doses were uniformly well tolerated by healthy research cats and kittens, and kittens with experimentally induced FHV-1 infections. A series of papers concerning this work is appearing. The only side effect of high doses of famciclovir reported to date is transient (reversible) loss of renal concentrating ability in a very small number of cats.

Despite the rigorous work from Davis, it is clear that much lower doses of famciclovir do indeed work *in vivo*, and only time will tell the optimal dose rate and dose interval for this drug in clinical practice. **Our evolving experience is that utilisation of higher doses on a mg/kg basis produces a faster response, and some feline patients only respond to high doses.** The drug is very well tolerated by cats. It is also remarkably well tolerated in human patients, including children and patients with HIV infection. Currently, the dose rate we recommend is largely dictated by the issue of cost, as the drug is expensive, and Maggs's recommended dose is just too expensive for most Australian owners, especially if a long course of therapy is required. **The authors therefore currently recommend a dose of 125 mg twice daily for adult cats (weighing 3.5 to 5 kg). For kittens, we recommend 25-40 mg/kg every 8 to 12 hours.** Such doses are more affordable in kittens, as they are smaller, and usually require shorter course of therapy for treatment of primary viral rhinosinusitis. A generic formulation of famciclovir (EZOVIR®) has recently become available in Australia. It is made by **Alphapharm**, a very reputable Australian company that makes a wide range of generic medicines. Famciclovir in this form is approx. 30% less expensive than the original formulation, and to date our experience has been that the generic drug is just as effective as the original. A similar generic formulation is available in the USA.

#### **OTHER THERAPUETIC CONSIDERATIONS**

Most cats with FHV-1 infections have secondary bacterial infection of either the skin, conjunctiva and/or sinonasal cavity. In our view, it is therefore prudent to treat concurrently with an antibacterial agent, and we

suggest the routine use of doxycycline monohydrate (5 mg/kg orally twice daily with food; Vibravet® tablets) for this purpose. Doxycycline has a broad spectrum of activity, with coverage of *Bordetella bronchiseptica*, *Pasteurella multocida* and obligate anaerobes, plus many *Staphylococcus* and *Streptococcus* isolates. Importantly, it is also active against the ocular pathogens *Mycoplasma felis* and *Chlamydophila felis*. Thus, if empiric therapy is undertaken, combination therapy with famciclovir and doxycycline should have all primary feline ocular pathogens “covered”, and this may be more cost-effective than obtaining a definitive diagnosis using the multiplex PCR for feline oculo-respiratory pathogens. The anti-inflammatory effects and metalloproteinase inhibitory action of doxycycline are useful also in this setting. Doxycycline monohydrate is well tolerated orally, and the tablets can be ground up in a pill crusher and mixed in with tasty food. The paste formulation is convenient to use in kittens, but is more expensive for adult cats, and pilling or crushing the medication in the food is more cost effective. A useful technique is to put the famciclovir and doxycycline into a single size 00 gelatine capsule, and give this immediately before meals using a dab of margarine (to help the capsule go down).

One of the key findings from David Maggs’s experimental work concerning FHV-1 in kittens and cats is that the number of goblet cells in the conjunctiva and therefore the concentration of mucin in the tear film is reduced by FHV-1 infection. Indeed, it takes the tear film many months to recover from a bout of viral conjunctivitis. Accordingly, there is benefit in using hyaluronic acid eye drops as adjunctive topical therapy for treating FHV-associated conjunctivitis and keratitis, to improve the quality of the tear film. Lacri-Lube® or chloramphenicol or tetracycline eye ointment twice daily represent good alternatives when owners cannot afford the expensive contemporaneously prepared medication.

Chronic snuffler cats may benefit from the administration of famciclovir in concert with *either* clindamycin, doxycycline or amoxicillin clavulanate, especially when high doses of famciclovir are used and the combination is continued for a protracted period, typically 2-3 months. Unfortunately, many cases only improve partially or transiently, presumably because some of the structural damage within the sinonasal cavity referable to FHV-1 is irreversible, resulting in ongoing secondary bacterial infections despite therapy.

Cats with unilateral or bilateral otitis media, which present with signs of peripheral vestibular disease (head tilt and/or Horner’s syndrome, or wide excursions of the head, respectively), appear also to benefit from combination therapy using famciclovir and one of the aforementioned antibiotics.

The authors strongly recommend routine treatment of kittens with viral respiratory disease with both famciclovir and doxycycline, especially if naso-ocular signs are present. In cases where Calicivirus is the primary agent, famciclovir will be of no benefit, and instead omega-interferon (Virbagen®; Virbac) is indicated, should the owners have sufficient financial resources (Interestingly, co-infection with both FHV-1 and Calicivirus is not uncommon). **Our great hope is that timely therapy using high doses of famciclovir will prevent many of the adverse long-term sequelae of FHV-1 disease including symblepharon, recurrent conjunctivitis and ulcerative keratitis, stromal keratitis, eosinophilic conjunctivitis, some cases of corneal sequestra, and especially the “chronic snuffler syndrome”,** where disruption of normal anatomy, mucosal barriers and viral persistence establish a lymphoplasmatic rhinitis, with variable secondary bacterial invasion. We suspect that a reduced prevalence of FHV-1 associated rhinosinusitis will also reduce the prevalence of invasive mycotic diseases of the nasal cavity, such as cryptococcosis, aspergillosis and sino-orbital disease due to *Neosartorya* species.

In cases where cats are prone to reactivation of FHV-1 associated signs with stress (such as boarding, parturition, lactation, hospitalisation, corticosteroid administration) it might be possible to prevent this happening by prophylactic use of famciclovir, or failing that, timely implementation of high dose therapy administered by the owners or carers at the first signs of viral recrudescence. Persistence of virus in the trigeminal ganglion means that “Herpes is forever”, although our experience had been that the frequency of recurrent episodes tends to decrease over a cat’s lifespan.

Finally, administration of famciclovir to queens starting immediately after parturition, and up until weaning, might decrease the frequency with which kittens are infected with FHV-1 in the perinatal period. This strategy, combined with timely administration of appropriate vaccines, may greatly reduce the frequency of FHV-1 disease in future generations of cats, especially pedigree cats kept in catteries and crossbred kittens raised in shelters.

### **Summary: Current recommendations**

1. For treating FHV-1 associated dermatitis, use topical cold sore cream (Zovirax® or Zolaten®) combined with famciclovir orally. Consider using an antibiotic orally for secondary *Staph* involvement e.g. amoxicillin clavulanate or doxycycline.
2. For treating conjunctivitis and keratitis due to FHV-1, use famciclovir in concert with doxycycline (5 mg/kg twice daily) plus topical hyaluronic acid eye drops or Lacri-Lube/chloramphenicol eye ointment twice daily.
3. For treating acute or chronic rhinosinusitis, or middle ear disease, combine famciclovir plus either doxycycline, clindamycin or amoxicillin clavulanate.
4. For most adult cats, 125 mg Famvir or its generic equivalent, orally twice daily is a reasonable starting dose, although some cats will do better if higher doses are used.
5. For kittens, use 30 to 50 mg/kg orally twice daily for acute viral respiratory disease, in concert with one of the aforementioned antimicrobials.
6. The duration of therapy is yet to be determined definitively, but generally courses should be in the order of 2 weeks for acute disease, and until the resolution of signs in chronic cases.

## NOTES

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# MYCOBACTERIAL UPDATES

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## PANNICULITIS DUE TO RAPIDLY GROWING MYCOBACTERIA

Rapidly growing mycobacteria (RGM) are a group of organisms that produce colonies on synthetic media within seven-days when cultured at 24°C-45°C. They are distributed ubiquitously in nature. RGM include the *M fortuitum* group (including *M fortuitum*, *M peregrinum*), the *M chelonae/abscessus* group (including *M chelonae* and *M abscessus*), the *M smegmatis* group (including *M smegmatis* sensu stricto, *M goodii* and *M wolinskyi*) and a variety of other species. The taxonomy of this group has been revised recently. Because of this, the word 'group' is used when referring to isolates recorded in early publications. RGM are strongly linked with localized infections of immunocompetent hosts. This is because they are well adapted to a saprophytic existence and inherently of low virulence. Thus, they do not produce disease unless a breakdown in normal defense barriers provides them with a portal of entry to a favorable tissue environment. Once introduced, RGM are generally constrained by a vigorous immune response that may or may not eradicate them, but is effective enough to prevent haematogenous or lymphatic spread. RGM can produce widely disseminated disease, but only in severely immunocompromised individuals. Mycobacterial panniculitis refers to a syndrome characterized by chronic infection of the subcutis and skin with RGM. This condition is quite common in cats, especially in Australia. It is seen less commonly in dogs, and also in Australian native animals such as Quolls and Tasmanian Devils. RGM replicate in tissues when introduced through a breach in the skin. This typically follows penetrating injury, especially when the wound is contaminated by dirt or soil. Preference of RGM for fat is a key factor in pathogenesis and results in a tendency for disease to occur in obese individuals and in tissues rich in lipid, such as the subcutaneous panniculus and especially the inguinal fat pad. Experimental infections cannot be induced in cats that do not have appreciable subcutaneous fat. Adipose tissue offers a favorable environment for survival and proliferation of RGM by providing triglycerides for growth or protecting organisms from the phagocytic/immune responses of the host. Initial reports suggested mycobacterial panniculitis was more common in warm humid climates; however cats from temperate regions, including parts of Australia, Canada, Finland and Germany, have been reported. In Australia, the *M smegmatis* group accounts for the majority of feline cases, whereas it is a much less common cause of equivalent infections in human patients. Infections tend to start in the inguinal region, usually following contamination of cat fight injuries, e.g. raking wounds inflicted with the hind claws. The infection may spread to contiguous tissues of the ventral and lateral abdominal wall and perineum. Penetrating injury by sticks, metallic objects and vehicular trauma may also give rise to these infections, as can cat and dog bite injuries contaminated with soil or dirt. Sometimes infections start in the axillae, flanks or dorsum. Early in their course, infections resemble catfight abscesses, but without the characteristic fetid odor and turbid pus. Instead, a circumscribed plaque or nodule is apparent. Later, there is progressive thickening of the nearby subcutis to which overlying skin becomes adherent. Affected areas become denuded of hair and numerous punctate fistulae appear, discharging watery exudate. Fistulae are intermingled with focal purple depressions (thinning of the epidermis over accumulations of pus). The 'lesion' gradually increases in area and depth, and may eventually involve the entire ventral abdomen, adjacent flanks or limbs. If cats are presented promptly for veterinary attention and the lesion confused with an anaerobic cat-bite abscess, surgical drainage and administration of a  $\beta$ -lactams is typically followed by wound breakdown and development of a non-healing suppurating tract surrounded by indurated granulation tissue. Some affected cats develop systemic signs, becoming depressed, pyretic, inappetent, losing weight and being reluctant to move. Occasional cats develop the hypercalcaemia of granulomatous disease, although this is rarely symptomatic. Surprisingly, other cats remain comparatively well despite extensive disease. Usually the problem remains localized to the skin and subcutis. Although adjacent structures such as the abdominal wall can be affected eventually, spread to internal organs or lymph nodes is very unusual. A tentative diagnosis can be confirmed by collection of pus or deep tissue specimens. This material is used to confirm the diagnosis using appropriately stained smears, histological sections and culture. A histological diagnosis is unnecessary if appropriate samples for cytology and culture have been procured. It is vital to give the

laboratory warning that mycobacterial aetiology is suspected so special procedures for processing can be adopted. **In our experience, samples of pus obtained from needle aspirates of affected tissues through intact skin provide the best specimens.** This material can be obtained from a palpably abnormal portion of the subcutis. The overlying skin should be carefully disinfected with 70% ethanol prior to obtaining material to preclude the isolation of saprophytic mycobacteria from the skin surface. It may be necessary to carefully move the needle in the subcutaneous space, while applying constant negative pressure, until a pocket of pus is encountered. **In dogs, the use of high resolution ultrasound can be helpful in locating suitable pockets of pus.** Aspirated fluid should be submitted for cytology and mycobacterial culture, or inoculated immediately into a commercially prepared mycobacteria culture bottle that is subsequently submitted to the laboratory. It is only necessary to suck a small amount of pus into the hub of the syringe. It is easiest to submit the entire syringe to the laboratory after replacing the needle with a sterile cover. Exudate from draining sinus tracts is heavily contaminated secondary invaders and represents an inferior sample. If deep biopsies are obtained, they should be triturated in brain heart infusion broth using a sterile mortar and pestle to produce a tissue homogenate suitable for cytology and culture. Smears prepared from aspirates or tissue homogenates should be stained using Diff Quik®, Gram stain and a modified acid-fast procedure (decolorizing with weak sulphuric acid for only three minutes; RGM are *not* as acid-fast as other mycobacteria). Cytology invariably demonstrates pyogranulomatous inflammation and it is generally possible to visualize Gram positive and/or acid-fast bacilli (AFB) in smears, although an exhaustive search may be required. Histologically, there is pyogranulomatous inflammation. AFB may be hard or impossible to find in Ziehl-Nielsen (ZN) stained tissue sections and are often located in lipid vacuoles. Some US dermatologists favour Fite's stain for detecting AFB in tissues. Tissue homogenates or pus should be streaked onto blood agar plates and a mycobacterial medium such as Lowenstein-Jensen medium or 1% Ogawa egg yolk medium and incubated aerobically at 37°C and 25°C. If available, the BACTEC system can also be utilized. Moderate to heavy growth of pinpoint, non-hemolytic colonies is usually detected after 2-3 days (occasionally longer) on sheep blood agar at 37°C. A useful method which can be used to differentiate RGM from contaminant flora is by primary isolation around antibiotic sensitivity discs (first generation cephalosporins or isoxazolyl penicillins) applied to the plate after inoculation. **There is great value in determining species identification and susceptibility data in every case, as this has a big impact on antimicrobial strategies.** Species identification can be carried out in a well-equipped veterinary bacteriology laboratory although it is often more convenient to send the strain to a Mycobacteria Reference Laboratory following primary isolation. Identification takes into account a number of phenotypic and biochemical features, although increasingly PCR using mycobacterial primers and sequence analysis of the resulting amplicon more rapidly provides a species identification. Minimum inhibitory concentrations (MICs) for ciprofloxacin, moxifloxacin, enrofloxacin, pradofloxacin, gentamicin, trimethoprim, clarithromycin, tigecycline and doxycycline can be determined easily using the Etest method. This methodology is less demanding than the 'gold standard' of broth microdilution. Antimicrobial susceptibility of clinical isolates can also be determined using disc diffusion methodology. The management of feline mycobacterial panniculitis continues to evolve in the light of clinical experience, availability of new anti-infectives and the development of new surgical techniques. There is great variation in the severity and extent of lesions from patient to patient. **Difficulty in making a prompt diagnosis is partly responsible for the chronicity, severity and refractoriness of these infections.** Briefly, treatment should commence with oral antimicrobial(s) (doxycycline, pradofloxacin/moxifloxacin and/or clarithromycin), initially chosen empirically, but subsequently based on *in vitro* susceptibility data. Sometimes long-term administration of such an agent or agents is sufficient to effect a cure, but in many severe cases it is eventually necessary to surgically resect recalcitrant tissues so that oral antimicrobial therapy will be able to eliminate the infection permanently. Given the extent and severity of the pathology in many of these cases, it is understandable that adequate levels of antimicrobials may not be achieved throughout all affected tissues and that in these cases the best chance for a successful outcome is to remove as much infected tissue as possible following preliminary drug therapy. Residual foci of infection can then be targeted by high concentrations of antibiotics achieved during and after surgery. Peri- and post-operative antimicrobial therapy is vital to ensure primary intention healing of the surgical incision. Ideally, the choice of antimicrobial should be based on culture and susceptibility testing. For empiric therapy, a combination of doxycycline (5 mg/kg orally twice daily using the monohydrate salt, or even higher doses if tolerated) plus moxifloxacin (10 mg/kg orally once a day compounded) will successfully treat most cases. **Pradofloxacin (3-6 mg/kg once daily depending on the formulation) has comparable efficacy to moxifloxacin, and will likely**

become the quinolone of choice for treating RGM (and other mycobacteria) once registered for cats and dogs. Clarithromycin is very effective for *M fortuitum* and *M chelonae/abscessus*, but does not work for over 70% cases with *M smegmatis* group infections. In my view, enrofloxacin is relatively contraindicated in these cases in cats because of the need for higher than routine doses and long courses, which amplify the risk of retinotoxicity. Pradofloxacin is safer likely far more effective than enrofloxacin in this setting. Until this drug becomes registered, the human drug moxifloxacin (10 mg/kg once a day) is recommended. Because of the propensity for bacterial resistance to develop during a course of therapy, it is prudent to combine drugs – for example moxifloxacin and doxycycline for *M smegmatis*/*M goodii* infections, and moxifloxacin and clarithromycin for *M fortuitum* and *M chelonae* infections. In cats where compliance is an issue, monotherapy with one drug is often sufficient to effect a cure, and for some drugs (e.g. doxycycline, clarithromycin), it is actually very rare for mutational resistance to develop during appropriate therapy. **It should be emphasised that rifampicin has no efficacy against RGM.** Clofazimine can be useful in certain refractory infections. Some centres are evaluating vacuum-assisted wound closure as an adjunct to the surgical treatment of extensive wounds created during debulking of extensive infections of the subcutis. Hyperbaric oxygen therapy may also have a place in the management of refractory cases, where such facilities are available. Intralesional and transdermal routes of administration may have a role to play in supplementing existing treatment regimens, especially in severe or refractory cases.

### FELINE LEPROSY SYNDROMES

The term feline leprosy is used to refer to a disease in which single or multiple granulomas form in the skin or subcutis in association with large numbers of acid-fast bacilli (AFB) which are non-culturable using standard methods. The condition was first recorded in the literature by Australian and New Zealand researchers in the early 1960s. Since then, the disease has been reported in Western Canada, the Netherlands, France, the UK, New Zealand and USA. Historically, the causative agent of feline leprosy was purported to be *Mycobacterium lepraemurium*. This bacterium causes murine leprosy, a systemic tuberculosis-like infection of rats. Cats are thought to contract *M lepraemurium* following bite injuries from infected rodents. *M lepraemurium* is a fastidious, slow-growing organism which, with difficulty, can be cultured from large inoculae on Ogawa's egg yolk medium under special conditions. Although a few investigators have successfully grown *M lepraemurium* from infected cats, the basis of ascribing this bacterium as the etiological agent of feline leprosy was dependent on transmission studies. **Interestingly, some cats appeared much more susceptible to experimental infection than others.** According to the literature, cats with feline leprosy are typically young adults (< 5 years-of-age), perhaps with a preponderance of males. Presumably these patient characteristics reflect the need for the cat to interact with a rat to become infected. The initial lesion is a focal granuloma of the subcutis. Owners become aware of solitary, or more commonly multiple, painless, raised, fleshy, tumor-like lesions, from a few millimeters up to 4 cm in diameter. These granulomas are freely movable over underlying tissues. Lesions can develop rapidly and when large, may ulcerate. Infection spreads to adjacent areas and may invade underlying tissues and drain to regional lymph nodes. Lesions can occur anywhere, but tend to be concentrated on the head and limbs. Small lesions are occasionally found on the tongue, lips and nasal plane. Lesions, even if multiple, tend to be initially concentrated in one region and have the propensity to recur following excision. Pathologically, feline leprosy was subdivided into lepromatous or tuberculoid forms based on the no. of AFB present (multibacillary v paucibacillary) and the host immunological response (lepromatous v tuberculoid). Because the causal mycobacteria are slow-growing organisms capable of intracellular survival, the histologic picture actually depends on the host's immune response. When this response is poor, lepromatous (multibacillary) disease develops with infiltration of the dermis with large sheets of 'incompetent' foamy macrophages containing enormous numbers of organisms. AFB are usually arranged in the cytoplasm of macrophages as dense parallel accumulations which displace the nucleus to an eccentric position. Lymphoid cells and plasma cells are virtually absent. If the host's immune response is more effective, histiocytic cells are accompanied by moderate numbers of lymphoid cells and plasma cells and multiplication of the organism is limited - the so-called tuberculoid response. AFB in smears and tissue sections appear as long slender rods. In smears stained with Romanowsky stains such as DiffQuik or Geimsa, organisms appear as **negative-staining bacilli**. In smears or sections stained with modified acid-fast stains such as ZN or Fite's stain, organisms take up the carbol fuchsin and are acid/alcohol fast. In humans, the presence of tuberculoid pathology is generally marker of disease in an immune-competent host and such infections are often initially

localised. In contradistinction, the presence of a foamy histiocytic infiltrate of the dermis and subcutis is observed almost exclusively in association with profound immunodeficiency, such as that seen with terminal HIV infection in human patients. Molecular methodologies have been used to investigate presumptive feline leprosy. Of eight cases of invasive or disseminated cutaneous mycobacterial disease investigated by Siobhan Hughes and colleagues using material collected largely from New Zealand cats, four were shown to have *M lepraemurium* infections. Of the remaining cases, one cat had a disseminated *M avium* infection, the aetiology in one cat was undetermined and in two cases infection was attributable to a novel mycobacterial species. This information encouraged a reappraisal of Australian feline leprosy cases, and subsequently this work has been extended to North America and Brazil. In Australia, cats were initially be divided into two groups based on the patients' age, lesion histology, clinical course and sequence of 16S rRNA PCR amplicons obtained from lesions. More recently, we have identified a new cohort of Australian cats from the Gippsland region of Victoria which were infected by a third novel mycobacterial species. The first group consisted of young cats (typically < 4-years) which initially developed localised nodular disease affecting the limbs. Lesions progressed rapidly and sometimes ulcerated. Sparse to moderate numbers of AFB were identified using cytology or histology, typically in areas of caseous necrosis and surrounded by tuberculoid inflammation. Organisms did not stain with haematoxylin and ranged from 2-6 µm (usually 2-4 µm). *M lepraemurium* was diagnosed based on the sequence of a 446 bp fragment encompassing the V2 and V3 hypervariable regions amplified from lesions using PCR and mycobacterial primers. The second group consisted of old cats (>9-years) with generalised nodular skin lesions associated with multibacillary lepromatous histology. Some cats initially had localised disease that subsequently became widespread; others had generalised disease from the outset. Disease progression was protracted, typically taking months to years, and skin nodules did not ulcerate. Microscopically, lesions consisted of sheets of epithelioid macrophages containing large to enormous numbers of AFB 2-8 µm (mostly 4-6µm) which **stained also with haematoxylin**. A single unique sequence spanning a 557 bp fragment of the 16S rRNA gene was identified in lesions from these patients. The sequence was characterized by a long helix 18 in the V3 region, suggesting the new species was likely to be a fastidious, slow-grower. The 16S rRNA sequence had greatest nucleotide identity with *M leprae*, *M haemophilum* and *M malmøense*, and contained an additional 'A' nucleotide at position 105 (the only other mycobacterial database sequence with the same extra nucleotide being *M leprae*). A very slow, pure growth of a mycobacterium species was observed on Lowenstein-Jensen medium (supplemented with iron) and semisolid agar in one case. The environmental niche of this new mycobacterium species has yet to be determined, although the preponderance of cases from rural or semi-rural areas of coastal NSW suggests it is a saprophyte found more commonly in these locations than in metropolitan environments. Since 2004, this novel mycobacterial species has been isolated from an additional 12 cases, including cats from Melbourne, Brisbane and Adelaide, and a human patient with cervical lymphadenomegaly. The third group consisted of over 12 cats, typically young adults (2 to 8 years), with lesions located on the head, cornea, conjunctiva or distal limbs, and lesions that were generally multibacillary and lepromatous. **The remarkable finding was that, with one exception, these cases were encountered in a very restricted part of rural Victoria.** The distribution of lesions is most compatible with a saprophytic organism being inoculated in tissues subsequent to cat scratch injuries. Widespread dissemination of infection (rather than local invasion) suggests decreased immunological surveillance permits the development of disease with an organism usually considered to have limited virulence. Feline leprosy caused by the novel NSW mycobacterial species, the Victorian novel species or more rarely *M lepraemurium*, may likewise represent a manifestation of deteriorating immune competence. For epidemiologic reasons, feline leprosy in young cats is usually caused by *M lepraemurium*, the novel NSW species is almost invariably seen in old (likely immunosuppressed cats) while the novel Victorian species can occur in either immune competent or immune defective cats. To make matters even more complex, recent work by Appleyard and colleagues has demonstrated a third mycobacterial syndrome in cats from western Canada and the USA (Idaho and Oregon) called 'feline multi-systemic granulomatous mycobacteriosis'. This disease is caused by a slow-growing taxa provisionally called *M visibile*. This species is capable of producing widespread dissemination to multiple internal organs, presumably in immune deficient cats. Sequence analyses demonstrate a number of nucleotide differences between *M visibile* and both *M lepraemurium* and the novel species reported by Hughes and colleagues. Diagnosis of the 'feline leprosy' syndromes is usually straightforward, provided that the clinician has a high index of suspicion for the condition. Needle aspirates, crush preparations of biopsy material and histological sections stained with ZN or similar methods contain easily demonstrable AFB surrounded by variable granulomatous to pyogranulomatous



inflammation. In DiffQuik stained smears, mycobacteria can be recognized by their characteristic ‘negative-staining’ appearance and location within macrophages and giant cells. Material should be submitted also for culture, because occasionally slowly-growing species such as MAC, *M haemophilum* and *M genavense* and the tubercle bacillus (*M bovis* or *M microti*) can produce an identical clinical presentation; in such cases optimal antimycobacterial therapy can be selected more readily on the basis of *in vitro* susceptibility results and information from the literature. In the majority of cases, however, conventional mycobacterial culture is negative due to the fastidious nature of the organisms and the exact aetiology can only be proven using PCR and sequence determination of gene fragments. PCR has the additional advantage of providing a rapid diagnosis. Fresh (frozen) tissue delivered to a mycobacterium laboratory with PCR facilities provides the optimal sample, although freeze-dried specimens may be more conveniently sent where tissues need to travel long distances. Sometimes PCR can be performed successfully on formalin-fixed paraffin-embedded material, although fixation conditions invariably cause some DNA degradation which may limit the success of the procedure especially when the tissue remains in formalin for a protracted period. Recently, Hughes and colleagues have developed specific PCR assays to diagnose infections due to *M lepraemurium* and the novel species; furthermore, use of a simple restriction enzyme digest allows these assays to distinguish *M visibile* strains also. Too few cases with a documented aetiology have been reported to provide definitive treatment guidelines. Although *M lepraemurium* and the novel species can be cultured *in vitro* with difficulty, it is currently not routine or reliable to isolate these organisms due to their slow growth and fastidious requirements. **Determination of *in vitro* susceptibility data for individual isolates is therefore not possible.** Only limited experimental studies have been undertaken to determine effective drug therapy for *M lepraemurium* *in vitro* or *in vivo* and as yet we have limited data only for the novel mycobacterial species. Portaels and colleagues found the minimum inhibitory concentration for rifampicin of two strains of *M lepraemurium* to be 4 and 8 µg/mL, levels that should be just obtainable *in vivo*. Other drugs shown to have activity against *M lepraemurium* *in vitro* include ansamycin compounds (rifabutin) and sulpha drugs. There is a good deal of clinical evidence that clofazimine has efficacy *in vivo*, while it is likely that clarithromycin would be also be effective based on its wide spectrum of activity against slow-growing mycobacterial species. The literature suggests that when *M lepraemurium* infection is diagnosed early, while disease is localized, wide surgical excision of infected tissues provides the best chance to simply and rapidly effect a cure. Aggressive resection techniques should be adopted, with *en bloc* resection of all lesions, and reconstruction of resulting tissue deficits using appropriate surgical techniques. Such an approach should be combined with adjunct antimicrobial therapy beginning a few days prior to surgery, so that effective levels of drugs are present in blood and tissues intra- and post-operatively to ensure primary intention healing. Clofazimine (at a dose of up to 10 mg/kg once daily orally; typically 25 to 50 mg every 24 to 48 hours) has the best reported success rate, although it is likely that combination therapy using two or more drugs will eventually prove superior. Drugs that could be combined with clofazimine include rifampicin and clarithromycin, although sulpha drugs, doxycycline, new fluoroquinolones such as moxifloxacin or pradofloxacin, or amikacin may in time also prove to be useful. In feline leprosy cases caused by novel mycobacterium species, we believe combination therapy using two or three of clofazimine (25 to 50 mg per cat orally every day or every other day), clarithromycin (62.5 mg twice daily), moxifloxacin (10 mg/kg once daily) or rifampicin (10 to 15 mg/kg per day) represents optimal therapy. However we are currently unsure of which will prove to be the best combination, and side effects in individual cats may affect which drugs are used in a given patient. Currently, we recommend a combination of rifampicin and clarithromycin as initial therapy. Other new agents such as linezolid may also have a place, although currently they are prohibitively expensive for most owners.

### **CANINE LEPROID GRANULOMA**

Canine leproid granuloma syndrome (CLGS), or canine leprosy, is the most common mycobacterial disease of dogs in Australia and Brazil. Although the causal organism has a worldwide distribution, its prevalence in other countries has not been well documented. Patients with this infection present with one or more nodules in their subcutis or skin, but are otherwise well. The condition was first described in a Boxer and a Bullmastiff from Zimbabwe in 1973, with similar reports from Australia appearing soon afterwards. Primary skin lesions consist of single or multiple, well circumscribed nodule(s). These **lesions can appear anywhere, although usually they are located on the head and typically on the dorsal fold of the ears.** The nodules are hard, painless, and vary in size from 2 mm - 5 cm in diameter. Small nodules are detected as hard subcutaneous lumps, while larger nodules

may show superficial hair loss. Very large lesions may ulcerate. Leproid granulomas are confined to the subcutis and skin and do not involve regional lymph nodes, nerves or internal organs. Affected dogs suffer no apparent systemic ill effects. This suggests that the causal organism has low pathogenicity or special prerequisites, such as a requirement for low temperature, which permits them to survive and multiply in superficial tissues only. Lesions can be disfiguring and cause irritation, especially when lesions are multiple and secondarily infected with *Staphylococcus intermedius*. CLGS has a wide geographic distribution, with cases recorded from coastal and inland regions of all states of Australia. The causal organism is likely to have a world-wide distribution, as the condition has also been reported in New Zealand, Zimbabwe, Brazil, Columbia, California and Florida. The authors are also aware of unreported cases of affected Boxers in New York state and Foxhounds in Georgia, USA. The condition would seem to be especially common in Australia and Brazil. Interestingly, there is a strong propensity for short-coated breeds to be affected, with Boxer and Boxer-cross dogs accounting for nearly half of the cases reported. Despite the fact that CLGS was first reported nearly 30 years ago, its aetiopathogenesis has not been fully elucidated. The initial report of the disease by Smith stated 'lesions appear suddenly, and are usually seen on dogs pestered by biting flies'. This might suggest that flies, or some other biting arthropod (such as midges or mosquitoes), inoculate mycobacteria from an environmental niche into susceptible tissues. The predilection for lesions to develop in regions favored by biting insect vectors, such as the head and particularly the ears, is consistent with this hypothesis, as is the overrepresentation of short-coated, large breed dogs (which are generally housed outdoors). Occasionally, clusters of dogs are infected simultaneously. Diagnosis is usually straightforward as the distribution of lesions (especially the propensity for the dorsal ear fold to be affected), coupled with the tendency for lesions to be multiple, particularly in an at-risk breed, is strongly suggestive of CLGS. Diagnosis can be confirmed by obtaining specimens of representative lesions for cytologic or histologic examination. DiffQuik-stained smears from needle aspirates typically demonstrate numerous macrophages with variable numbers of lymphocytes and plasma cells and lower numbers of neutrophils. Usually few-to-moderate negatively-stained, medium-length bacilli can be detected within macrophages or extracellularly. Histologically, lesions within the subcutis and dermis consist of pyogranulomas composed chiefly of epithelioid macrophages, Langerhans-type giant cells with scattered neutrophils, plasma cells and small lymphocytes. The number and morphology of AFB in ZN-stained section is highly variable from case to case. Currently, it is impossible to confirm the diagnosis by culture, as the *in vitro* growth requirements for this fastidious organism have not been determined. A negative culture, however, can exclude other mycobacterial etiologies. It is vital to thoroughly disinfect the skin surface prior to obtaining specimens for culture, as saprophytic mycobacteria can easily be cultured erroneously from dirt present on canine skin. PCR methodologies using both universal and mycobacterial primers designed to amplify regions of the bacterial 16S rRNA gene have been performed on CLGS specimens from dogs. Using sequence capture PCR for paraffin-embedded specimens and nested PCR on DNA from fresh tissue specimens, a novel PCR product has been identified with identical sequence over a 350 bp region. Analysis of the partial 16S rRNA sequence supports the notion that the novel species is a fastidious, slow-growing mycobacterium. In total, molecular methodologies identified this proposed novel mycobacterial sequence in material from in excess of 25 Australian cases of CLGS indicating that the species represented by this sequence is probably the principal causative agent of CLGS. Our continuing experience, and those of colleagues in California and Brazil, supports this contention. Interestingly, the species represented by this sequence has never been recorded from mycobacterial granulomas affecting the skin or subcutis of cats, horses, people, or other non-canine mammalian species. Hence, there is thought to be no public health risk to the owners of affected dogs. Very little has been written concerning the treatment of CLGS. Many cases are self-limiting, with the nodular skin lesions regressing spontaneously with time, typically within 1 to 3 months of appearing. The stated time frame is based on our experiences consulting with veterinarians in relation to cases diagnosed histologically; by the time the sections are submitted, processed, reported on and we establish a dialogue with the clinician, lesions are often already starting to regress, either spontaneously, or in response to antimicrobials (useful for secondary *Staph intermedius*, but with unlikely efficacy for mycobacteria). This 'self-cure' occurs as a result of an effective cell-mediated immune response mounted by the patient. In cases with a limited number of lesions, surgical excision can be curative and provides material with which to confirm the diagnosis histologically and using PCR. In other cases, however, the infection progresses to produce chronic, disfiguring lesions that may persist indefinitely. Limited information suggests that treatment with conventional antimicrobial regimens using  $\beta$ -lactam drugs or doxycycline (as monotherapy) fails to have a significant impact on the course of infection, although these drugs

may be of some benefit by effectively treating secondary pyogenic infections. One report concerning two dogs from Brazil suggested topical antibacterial treatment and orally administered rifampicin may be effective. Our experience treating ‘canine leprosy’ suggests that this infection responds to therapy with combinations of antimicrobial agents known to be effective against non-tuberculous mycobacteria, including rifampicin, clarithromycin, clofazimine, moxifloxacin and doxycycline. Based on our evolving experience, a combination of rifampicin (10 to 15 mg/kg PO every 24 h) and clarithromycin (15 to 25 mg/kg PO total daily dose; divided and given every 8 to 12 h) is currently recommended for treating severe or refractory CLGS cases. Treatment should be continued until lesions are substantially reduced in size (typically for 4 to 8 weeks) and ideally until lesions have resolved completely. It is prudent to monitor hepatic function periodically during treatment, as rifampicin may cause hepatotoxicity in some patients. A topical formulation containing clofazimine in petroleum jelly may be used as an adjunct to systemic drug therapy, and recently we have been using silver sulfadiazine topically in cases with relatively superficial lesions. Intralesional therapy also may be worth trialing in future refractory cases.

### **LOCALISED INFECTIONS DUE TO MYCOBACTERIUM ULCERANS**

*Mycobacterium ulcerans* is the causative agent of Bairnsdale/Buruli ulcer, a chronic localised infection of the skin and subcutis of human patients typically associated with (often extensive) necrotizing skin ulcers with undermined edges. However, pre-ulcerative lesions such as small red papules or painless sub-cutaneous nodules have also been described. *M. ulcerans* is closely related to *Mycobacterium marinum*, the “fish tank” or “swimming pool bacillus”, which also causes infections of the skin and subcutis when contact with contaminated water or soil occurs in association with penetrating injury or maceration of the skin. The extensive tissue destruction characteristic of ulcerative *M. ulcerans* infections is caused by an unusual polyketide-derived macrolide, called mycolactone, with cytotoxic and immunosuppressive properties. The genes encoding the mycolactone biosynthetic pathway are located on a 174 kb plasmid which is likely to have been acquired by horizontal gene transfer during the evolution of the *M. marinum*/*M. ulcerans* complex. Whereas infections due to *M. marinum* occur in almost all geographical regions around the world, disease due to *M. ulcerans* is restricted to certain highly localised geographical regions, presumably because the causal organism has a restricted environmental niche, which has yet to be determined, but can be inferred from the structure of the *M. ulcerans* genome. The disease is most prevalent in West Africa where it is referred to as Buruli ulcer (<http://www.who.int/buruli/en/>), whereas in Australia it occurs mainly in regions of coastal Victoria and Queensland, where it is known as Bairnsdale or Daintree ulcer. Non-human cases of *M. ulcerans* infection have only been reported from Victoria, mainly in marsupial species such as koalas (*Phascolarctos cinereus*), common ringtail possums (*Pseudocheirus peregrinus*) and a long-footed potoroo (*Potorous longipes*). A single case has been described in an alpaca (*Vicugna pacos*). Recently, we have been involved in the diagnosis and/or therapy of a cat, two horses and two dogs with *M. ulcerans* infections. PCR is exceedingly helpful for rapid diagnosis, although the organism does grow on synthetic media. Treatment involves judicious excision of infected tissues, followed up with combination therapy using rifampicin, clarithromycin or a fluoroquinolone. In one horse, cryotherapy proved to be effective in resolving the lesion in the absence of systemic antimycobacterial therapy.

## NOTES

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# **MYSTERY DERMATOLOGY CASE: NOVA**

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## **SIGNALMENT**

Nova is a 15 year old gelding Westphalian horse

## **HISTORY**

Nova was purchased about 1 year ago by the current owner. Within the past 3 weeks, Nova has developed several small circular white spots all over the body. The only other medical condition for this horse is a lameness issue which has been treated with Adequan and periodic steroid injections. Nova has not received either of these two injections within the past month. He is current on vaccinations. Nova is routinely dewormed with ivermectin. According to the owner, the skin lesions do not appear to be pruritic.

## **PHYSICAL EXAMINATION**

On physical examination, Nova has several small, multifocal, circular areas of partial to complete alopecia with leukotrichia and leukoderma. The skin lesions are predominately on the face at the base of the ears and on the ventrum (especially around the prepuce). However, a couple areas of leukoderma and leukotrichia are also present on the trunk and at the base of the tail. Nova has a few nodules (2 cm by 2 cm) on the lateral aspects of the neck and on the cranial chest.

## **DIFFERENTIAL DIAGNOSES**

## **DIAGNOSTIC TESTS**

## **DIAGNOSIS**

## **TREATMENT RECOMMENDATIONS**

## **FOLLOW UP**

## **SELECTED READINGS**

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3. Montes LF, Abulafia J, Wilborn WH *et al.* (2003) Value of histopathology in vitiligo. International J of Dermatol 42(1): 57-61.
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## NOTES

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# INTERESTING EQUINE CASES

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## CASE 1

'Doc'

10 year old Quarter Horse gelding

Weight: approx 500 kg

### History:

Two months prior to examination at the Veterinary Medical Teaching Hospital, University of California, Davis (VMTH-UCD), the horse developed ventral edema, limb edema, draining purulent material from the ventral abdomen and inguinal region. Relevant bloodwork showed an increased creatinine kinase (CK) (512), slight hypoglycemia (65), slightly decreased bicarb (23), WBC 10,400, RBC 6.88, and a degenerative left shift (78 bands). Several days later, blood work showed a leukocytosis of 19,400 with a neutrophilia of 16,684 and anemia of chronic disease (PCV = 26.0%), and hypoglycemia (47 mg/dl). Over the next month the referring DVM documented a rise in the *Corynebacterium pseudotuberculosis* titer of 1:8 to 1:2048

The referring DVM treated the horse with trimethoprim-sulfa (TMS), then when no response was noted, changed to a combination of ceftiofur and rifampin. A dramatic improvement was then noted in the edema. Some febrile episodes persisted, and either after the first antibiotics were started, or just before, small encrusted nodules were noted, first on the face then (after all antibiotics were discontinued) these crusts progressed to involve much of the trunk. These lesions progressed to alopecia with scaling. The horse is no longer febrile, and the lesions are not painful or pruritic, although the horse is more lethargic than usual.

### Physical examination:

Trunkal and facial scaling and alopecia.

### Differential diagnoses:

Pemphigus foliaceus (drug-induced or naturally occurring)

Other drug eruptions

Equine sarcoidosis ('chronic granulomatous disease')

Infectious (dermatophyte, pyoderma)

### Diagnostic tests:

CBC: within normal limits (WNL)

Chemistry: slight hyperglycemia, slight hyperglobulinemia, slight decrease of CK

Cytology of abdominal fluid: WNL

*Corynebacterium pseudotuberculosis* titer 1:2560

Histopathology: mild multifocal chronic eosinophilic and lymphoplasmacytic perivascular superficial and deep dermatitis with dermal mucinosis and fibrosis. No active vasculitis.

Comment: inflammatory dermatitis, possible drug reaction



Working diagnosis:

Drug reaction due to antibiotics. Possible cutaneous reaction to internal abscesses/continuing nidus of infection?

Treatment:

oral dexamethasone (20 mg/day) for 5 days, followed by 4 weeks of prednisolone (500mg/day) *per os* then 500mg every other day.

Result:

Rapid improvement in alopecia and scale with resolution of lethargy. Some diarrhea noted which resolved with eventual discontinuing of prednisolone.

**CASE 2**

‘Scotty’

9 year old Welsh pony stallion

Wt approx 250 mg

History:

The pony developed crusts/scaling on tail head 3 months prior to examination at the VMTH-UCD.

These progressed to ulcers and involved to rear of legs, sheath, and crusts in the mane. The horse is generally mildly to moderately pruritic. Ulcerative lesions on muzzle were present for 1 week. Referring DVM performed a bacterial culture which showed ‘mixed growth’ and a fungal culture which was negative. The referring DVM has treated Scotty with oral trimethoprim-sulfa, phenylbutazone and a gentocin-containing ointment without any effect noted on the lesions.

Physical examination:

Crusts and ulcerations caudal hind legs and tail base

Ulcers on muzzle, diffuse ulcers orally and on tongue

Mild ulceration periocular OU

Submandibular lymphadenopathy

Differential diagnoses:

Drug eruption/erythema multiforme

Bullous pemphigoid (rare in horses – 2 seen at UC Davis in 8 years)

Pemphigus vulgaris (very rare – not well documented in horses)

Diagnostic tests:

CBC: WNL

Chemistry: slightly low BUN

Fungal culture: saprophyte

Bacterial culture: small number of colonies of *Staphylococcus aureus*

Histopathology: severe multifocal suprabasilar vesicular dermatitis with numerous acantholytic cells and rafts and multifocal basilar and superficial keratinocyte single cell necrosis.

Immunohistopathology [Grateful thanks to Dr. Theiry Olivry]:

Direct immunofluorescence: some skin fixed IgG between keratinocytes and on the membrane of sebocytes and sweat glands. There was also a high titer of antikeratinocyte serum IgG (titer on canine lip: 1:320). This supports the diagnosis of PV.

Indirect immunofluorescence: confirms the presence of some circulating antikeratinocyte IgG autoantibodies using equine lip as substrate. The final titers are 1:80 (IgG - not very high), < 1:10 IgA and < 1:10 IgM. The pattern is compatible with that of an anti-Dsg3 response (i.e. "bottom heavy" pattern).

Testing of pony's serum for the presence of antibodies directed specifically against equine Dsg is pending.

Working diagnosis:  
Pemphigus vulgaris

Treatment:  
Dexamethasone 27 mg IM once, then started on prednisolone (0.8 mg/kg/day) *per os*.

Response and clinical course:  
Dramatic improvement of skin lesions, oral and lingual ulcers static. After 2 weeks, laminitis necessitated reduction of prednisolone dosage to 0.4mg/kg. Cutaneous lesions recurred, much more severe. In an attempt to control the skin disease and to avoid/decrease the dosage of corticosteroids, azathioprine (1mg/kg/day x 1 month *per os*), dapsone (3mg/kg/day x 2 weeks *per os*), and the gold salt aurothiomalate 1mg/kg q 7 days x 3 weeks IM) were all used successively. In addition, triamcinolone spray (0.015% ) and cream were applied to the affected areas, and a lidocaine gel/oral rinse applied to the oral cavity for comfort.

60 days into the treatment, the pony developed a corneal ulcer OD. According to the veterinary ophthalmology service at UC Davis, horses are not prone to develop corneal ulcers due to corticosteroid treatment. The human literature states that desmoglein 3 is not found in the cornea – the few corneal ulcers in PV cases in humans were felt to be due to the patient rubbing at the ulcerated skin periocularly. The corneal ulcer responded well to treatment.

Because of concern of difficulty apprehending food, endoscopy was performed on the pony's throat and esophagus, approximately 70 days into the treatment. Results were normal. The pony had several bouts of colic that were managed medically.

Despite the medication and treatment noted above, the pony's condition deteriorated and it was euthanized 3.5 months after being initial examination at the VMTH-UCD.

Necropsy:  
Skin: confirmation of PV. Ulcers extending from oral cavity into esophagus and the gastric cardia. Laminitis, bronchopneumonia and severe body wasting were also present.

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### **Case 3**

'Dunny'

6 week old Quarter Horse colt

#### History:

Alopecia noted for two weeks prior to examination at VMTH-UCD. Initially pruritic, referring DVM treated with diphenhydramine, topical and oral (25mg bid *per os*). No other horses, including sire and dam, have alopecia, but all are somewhat pruritic – owner attributes this to flies. No longer pruritic. Alopecia started on cranial thorax, now has affected neck and muzzle. Colt and its sire are HERDA (hereditary equine dermal asthenia) carriers.

#### Physical examination:

Patchy alopecia areas over cranial thorax, right shoulder, neck, muzzle and hocks. The hocks have a 'splash' pattern. The alopecic areas appear to have fine short hair growing within the spots except for a 3x5 cm area over the caudal gastrocnemius tendon. No excoriations noted.

#### Differential diagnoses

Alopecia areata

Dermatophytosis

Demodicosis

Telogen effluvium

#### Diagnostic tests:

Cytology: WNL

Trichogram: normal

Dermatophyte culture: negative

Histopathology: Skin (shoulder) mild chronic superficial perivascular dermatitis with ischemic change of collagen, focal microhemorrhage, mild multifocal serocellular crust, mild diffuse epidermal hyperplasia with hyperkeratosis

Skin (neck): mild chronic superficial perivascular dermatitis with ischemic change of collagen, multifocal microhemorrhage, mild diffuse epidermal hyperplasia with hyperkeratosis

Comment: In the sections of skin examined there were no obvious signs of alopecia as the hair follicles at varying stages of development were present.

#### Working diagnosis:

telogen effluvium

#### Clinical course:

Twenty days after initial examination at VMTH-UCD, owner reported that all the hair had regrown

#### **Case 4**

'Dusty' and 'Eeyore'

Miniature donkeys

(Thanks to Dr. Meredith Boulay, Norfolk MA for this case)

#### History:

Eeyore had no problems for the first year of ownership, then Dusty arrived January 2006. Eeyore, then Dusty, became very pruritic and developed moderate to severe scaling. DVM treated with ivermectin, various topical for lice, TMS, dexamethasone; none of these effected a change in the donkeys' condition.

#### Diagnostic tests:

Multiple skin scrapings and two dermatophyte cultures: negative

Histopathology: moderate, diffuse, chronic, superficial perivascular, hyperplastic lymphocytic dermatitis. A recut was sent to Cornell: WNL

#### Clinical course:

June 06 – haircoats returned to normal

November 06 – process restarted, and continued to cycle non-seasonally.

Treatment during this time of 3 lime-sulfur dips – minimal response

October 2007– regrew their coats again, minimal to no seborrhea

Question: What do/did these donkeys, and donkeys with similar signs, have??

## NOTES

This image shows a single sheet of white paper with horizontal blue or grey ruling lines. The lines are evenly spaced and run across the width of the page. There are approximately 20 lines visible. The paper has a slight shadow on the right side, suggesting it's resting on a surface. There is no handwriting or other markings on the paper.

## EQUINE CASES - THREE CRUSTY HORSES

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**Case 1:** 4 year old Thoroughbred Mare

**Presenting Complaint:** Crusty skin lesions

**History:** 3 month history of patches of crusting skin lesions on the neck progressing to mane and chest. Initial treatment by the owner with betadine scrub seemed to help, but within 2-3 weeks the lesions spread throughout the neck area and the mane appeared to be flaky as well. Treatment was changed to chlorhexidine shampoo, but lesions continued to progress. Treatment with trimethoprim/sulfamethoxazole (960 mg) 16 tablets orally q 12 hours for 7 days did not result in improvement. After a treatment with 6 mg of dexamethasone IV for 2 consecutive days the lesions dried up and reduced in size. A week prior to presentation the referring veterinarian had found cocci shaped bacteria on direct skin impression cytology and restarted the TMS treatment. At presentation no improvement had been noticed.

**Physical Exam:** temperature 100.2 F, pulse 35/min, respiration 16/min, weight 1160 pounds. Large areas of crusting dermatitis most intensely along the neck including the mane and ventral chest. Legs appeared to be unaffected. Edema along the ventral chest was present. Hair in affected areas epilated easily.

**Differential Diagnosis:**

**Diagnostic Tests:**

**Diagnosis:**

**Treatment:**

**Comments:**

**Case 2:** 21 year old Arabian Stallion

**Presenting Complaint:** Crusts and alopecia

**History:** 15 year (!) history of intermittent crusting of the skin and subsequent hair loss in the affected areas. The lesions waxed and waned and were not associated with any particular season, medication or diet. Several antibacterial and antifungal shampoos had been tried without improvement. Recently four ivermectin treatments had been given two weeks apart and a three day course of steroids: a dexamethasone injection followed by 3 days of dexamethasone powder (both doses unknown) but no improvement was noticed. The owner described that the lesions would start with moist and matted hair and about 2-3 days later start to dry up, become scaly followed by hair loss in the affected areas. Three months after each outbreak the hair would be fully grown back in the affected areas. No pruritus was reported and other horses in contact were unaffected. The past winter had been the worst ever with large lesions present at all times. The diet included alfalfa and timothy hay with a commercial sweet feed.

**Physical Exam:** Temp: 98.8F, pulse 40/min, respiration 12/min, weight 840 lbs. Skin lesions were confined to the neck and dorsal thoracic area. Lesions consisted of either large patches of matted hair (newer lesions) or very large epidermal collarette type lesions with thick scales peeling of the underlying skin with hair shafts pulling back with the thick scales, leaving alopecic patches with fine scaling behind. Lesions did not appear painful.



**Differential Diagnosis:**

**Diagnostic Tests:**

**Diagnosis:**

**Treatment:**

**Comments:**

**Case 3:** 21 year old Quarter horse gelding, "Pinto"

**Presenting Complaint:** mildly pruritic, crusting dermatitis

**History:** Two year history of crusting skin lesions starting in the axillary region with hive like lesions that oozed serum-like fluid and then dry up and crust over within a few days. Mild pruritus was noticed after the lesions appeared. A three month treatment with griseofulvin did not improve the lesions. Shampoo treatment appeared to help with the crusting and the pruritus and potentially explained while during the previous winter lesions were worse (bathing seldom possible during the cold weather). An ELISA test by Pet Allergy Laboratory, Austin TX revealed mostly negative or borderline reactions except for Goldenrod, Russian thistle (and Beet Pulp, Cottonseed). Skin biopsies by the referring veterinarian revealed lymphocytic mural folliculitis with negative special stains. A diet change from alfalfa hay and oats to a pelleted commercial food did not improve the skin lesions.

**Physical Exam:** Temp: 99.8F, pulse 48/min, respiration 24/min, weight 1000 lbs. Generalized patchy alopecia with fine scaling/ crusting including mane and tail but sparing the legs.

**Differential Diagnosis:**

**Diagnostic Tests:**

**Diagnosis:**

**Treatment:**

**Comments:**

## NOTES

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# NONHUMAN PRIMATE DERMATOLOGY: RESEARCH AND ADVANCES

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## INTRODUCTION AND KEY POINTS

The study of dermatologic diseases in nonhuman primates (NHP) has been neglected almost entirely by veterinary dermatologists. The scientific literature regarding this subject is disparate and unorganized. In “Nonhuman primate dermatology: a literature review” in 2009, this author attempted to gather wide-ranging case reports and studies into a more cohesive format to provide a baseline reference point for the veterinary dermatologist interested in the study of NHPs.<sup>1</sup> The aim of this discussion is to provide encouragement to members of the American College of Veterinary Dermatology (ACVD) to participate in NHP case work and research in conjunction with the primatologists of the Association of Primate Veterinarians (APV). The following key points will be discussed:

- Basic Taxonomy
- Health requirements and safety precautions for the dermatologist preparing to work in a facility housing NHPs
- Update on NHP dermatologic research in the past two years focusing on NHP alopecia
- Recommended reading

## TAXONOMY

The order Primata includes more than 200 species divided into two sub-orders; the Haplorhini (tarsiers, Old World and New World monkeys, apes and humans) and the Strepsirhini (bushbabies, lorises and lemurs). The Groves classification system is the most commonly used and can be referred to for a detailed primate taxonomy.<sup>2</sup> Rhesus (*M. mulatta*) and cynomolgus (*M. fascicularis*) macaque species are the most commonly studied NHPs in biomedical research.

## HEALTH AND SAFETY PRECAUTIONS

Because of the close phylogenetic relationship between non-human primates and humans, zoonotic and reverse-zoonotic diseases caused by a number of pathogenic organisms are of concern. Essential aspects of an institution's Occupational Health and Safety Programme (OHSP) include: health screening of animals and personnel, risk assessment of infectious and non-infectious hazards and personal protective equipment requirements determined by the primate species, health status and work environment.<sup>3</sup>

- Tuberculosis: TB testing (intradermal PPD) is typically required every 6 months to work with NHP patients.
- Recommended (sometimes required depending on institution) vaccinations for clinicians: Measles, Rabies, Hepatitis A, Hepatitis B
- Personal protective equipment can range from dedicated clothing, footwear and gloves to a more typically encountered higher level of barrier protection including mask, hat, goggles and/or face visor. This can make dermatologic examination uncomfortable and sometimes difficult.
- Engineering controls (crush backs and tunnel catching systems), as well as chemical controls (sedation typically with ketamine 10-15 mg/kg IM) are used to prevent bites and scratches, as well as exposure to bodily fluids.

The most significant zoonotic concern for clinicians and keepers is “Herpes B Virus (BV)”. Formerly named *Cercopithecine herpesvirus 1*, it has recently been renamed by the International Committee on Taxonomy of Viruses in the 2008 overhaul of the classification system as *Macacine herpesvirus 1* (McHV-1).<sup>4</sup> In its reservoir hosts (*Macaca* genus), this organism produces a mild clinical infection similar to HSV in humans followed by latency in sensory nerve ganglia. Typical clinical signs are erythema and vesicles on mucocutaneous junctions. Transmission to its non-natural hosts (humans, African monkeys and New World monkeys) usually results in a fatal encephalitis. Despite the development of specific pathogen-free (SPF) macaque colonies, due to issues with reliability of assessment of viral status by antibody assay, all macaques should be considered potentially infected.

## **UPDATE ON NHP DERMATOLOGIC RESEARCH- FOCUS ON ALOPECIA**

The majority of efforts in recent dermatologic investigations in captive NHP species have focused on the causes of alopecia. The disproportionate amount of research energy being devoted has been spurred by regulations promoting the psychological well-being of captive NHPs and a recent focus by USDA regulators on alopecia. A default diagnosis of psychogenic alopecia (trichotillomania) is often presumed on inspections of facilities without an evidence-based diagnostic approach. As in companion animal patients, alopecia can be either a primary or secondary lesion; and numerous factors and causes have been reported in the NHP.<sup>5-12</sup> An excellent comprehensive review article published in 2009 (Novak MA and Meyer JS) summarized causes of alopecia including: seasonal variation, aging, nutritional and hormonal imbalances, immunologic and genetic factors, bacterial, parasitic, fungal, allergic and psychologic factors.<sup>13</sup> Recommendations for an organized diagnostic assessment of cases were made as well as management strategies.

The researchers of the New England Primate Research Center (NEPRC), Harvard Medical School worked in 2010 on clinical and dermatopathologic evaluation of different presentations of alopecia in a large colony of Indian-origin rhesus macaques.

In the first of the two alopecia studies undertaken by the NEPRC group, a group of rhesus macaques with varying degrees of alopecia were biopsied to assess underlying pathology. Twenty-five affected individuals were sampled along with 11 control animals chosen for normal hair coats. Twenty-four of the affected animals had clinical dermatitis associated with the alopecia. Alopecia was associated with perivascular mononuclear cell infiltrate, acanthosis, hyperkeratosis, and edema. Immunohistochemical and metachromatic stains revealed increased numbers of mast cells, CD3+ lymphocytes, CD 163+ histiocytes and dendritic cells. The immunophenotypic analysis showed a statistically significant difference between the affected and control patients. Complete blood count, chemistry panels, cortisol levels and thyroid function tests showed no significant differences between affected and unaffected animals. Pathologic findings in this study led to the conclusion that the affected patients likely had a chronic hypersensitivity reaction or atopic dermatitis-like disease.<sup>14</sup>

The follow-up study focused on a subset of alopecic rhesus macaques with low grade alopecia localized to the forearms or lower legs. This clinical subset (n=17) had no evidence of excoriation or dermatitis but anecdotally caretakers had reported hair picking and pulling behaviors in the affected anatomic areas. Skin scrapes, surface cytologies, and bacterial and fungal cultures revealed no evidence of parasitism, bacterial or fungal infection. Biopsies were taken from affected and unaffected areas on the same limb. Skin biopsies demonstrated little difference between alopecic and non-allopecic areas. Features of trichotillomania in humans<sup>15</sup> were rarely noted: increased catagen follicles (n=2), reduced numbers of hair follicles (n=1), trichomalacia (n=3), and intrafollicular hemorrhage (n=2). No bulbar inflammation or folliculitis was noted and no perivascular inflammation, acanthosis or dermal edema were noted as compared with the patients in the previous study. Mast cell and CD3+ lymphocyte counts were the same in affected and unaffected skin samples. The authors proposed that this focal presentation of self-inflicted limb alopecia, unlike the presentations in the prior study, was most consistent with a psychogenic self-inflicted alopecia (accepted for publication January 2011, Kramer, J, Mansfield, KG, Simmon, J, Bernstein, JA. Psychogenic alopecia in rhesus macaques presenting as focally extensive distal limb alopecia. Comparative Medicine.)

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## NOTES

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# **CYTOLOGY OF CUTANEOUS NEOPLASIA – *WHAT IS NORMAL?***

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Since exfoliative cytology is most commonly used in the diagnosis of cutaneous inflammatory or neoplastic processes, it is necessary for the dermatologist to be experienced in the identification of abnormal cell types. It is equally important, however, that he or she be proficient at the identification of normal (non-inflammatory, non-neoplastic) cell types which might be found in a sample collected from the skin. Aspirates, coring cytologic preparations, and scrapings of biopsies prepared from cutaneous lesions will contain these normal cell types as individual exfoliated cells and tissue fragments. If the individual evaluating the cytologic specimen cannot recognize normal cell types, how can he or she expect to identify a dysplastic or neoplastic process?

Cytologic identification of a normal organ from exfoliated cells is often difficult due to the absence of characteristic patterns of histologic structure which help to identify the tissue. Individual cells aspirated from an organ may look quite different from the same cells seen in histologic section, but sometimes are strikingly similar! 'Normal' cytology must be learned by study of histology textbooks, collection of aspirates, corings, and scrapings from normal tissues, comparison of cytologic preparations with the histology of the same organ, and experience. The ability to make a diagnosis in a pathologic process is firmly based upon a clear understanding of both normal and abnormal cytologic structure and function. Many skin tumors can be diagnosed by seeing the similarities to the corresponding normal cell type.

Normal skin is composed of a thin epidermal layer of stratified squamous cells, with a thicker dermis; there is a layer of melanocytes immediately below the epidermal surface. Langerhans cells (antigen-presenting cells) will be found in the epidermis and may sometimes be found on cytologic preparations if they are increased in number. In the dermis, collagen, hair follicles, sebaceous glands, and apocrine glands will be found. Below this is the subcutaneous adipose tissue, and below that the muscle. It is nearly impossible to 'aspirate' the normal epidermis, but one may obtain a group of cells from the stratum corneum whenever one aspirates any mass or lesion and goes through the skin, and other cutaneous 'incidental' cell types can also be obtained on the way through the dermis.

A scraping of the epidermis will consist principally of superficial keratinized squamous epithelial cells called 'squames'. The keratinized cells from the stratum corneum usually exfoliate singly or in small sheets, and have the shape of irregular polygons. In dogs with pigmented coats, a sprinkling of brown melanin pigment may normally be seen in the squamous cells. In a fully keratinized cell, the nucleus is pyknotic or absent, having undergone karyolysis. A pale area in the center of the polygonal cell is the ghost-like area where the nucleus was prior to karyolysis. Sometimes squames will fold into small cylinders or tubes; these surface cells are sometimes found on cytologic aspirates of deep structures, since they can be carried in on the needle tip from the skin surface. If a scraping of skin is obtained from areas deep within the epidermis, less mature cells will be found. These basaloid cells obtained from lower within the epidermal layer are cuboidal, have more deeply basophilic basophilic cytoplasm, are likely to occur in clusters, and have a N/C ratio of 1/2 to 1/3; they are generally obtained only from imprints or scrapings of biopsies.

Scrapings from the dermis will sometimes contain adnexal structures, and aspirates or scrapings that include tissue from the subcutaneous cell layers will show fat and sometimes a muscle fiber from the deeper tissues. There may be clusters of secretory cells from sebaceous glands; mature sebaceous cells are filled with small secretory droplets and have centrally-located, uniformly-sized nuclei. Clusters of apocrine glands will only rarely be found as small clusters of cuboidal or short columnar cells, sometimes with blue granules in the cytoplasm. Heavily-pigmented melanocytes may very rarely be found from the dermal/epidermal junction. Fat droplets and adipocytes from the subcutaneous tissue may also be noted.

## MISCELLANEOUS CELLS

Fibrous connective tissue - Fibrocytes are often seen in scrapings from the dermis and subcutaneous tissue, as is bright pink collagen in which the fibrocytes are embedded. Fibrosis is the formation of fibrous connective tissue as a response to chronic insult, and is sometimes seen in chronic inflammatory processes, especially if there is scar formation. Fibrous connective tissue cells are so strongly tissue-bound that they are only rarely found in cytologic aspirates or imprints; they are commonly found in scrapings, however. Young cells, or fibroblasts, are spindle-shaped with long, tapered, delicate to wispy stellate cytoplasmic processes and an ovoid nucleus with indistinct nucleoli. Reactive fibroblasts have enlarged, plump nuclei and coarse nuclear chromatin, with a visible nucleolus, and occasional binucleated cells may be seen. The cytoplasm of the reactive fibroblast is often quite basophilic, with the degree of basophilia being directly proportional to the activity of the cell. The more reactive fibroblasts contain more endoplasmic reticulum in their cytoplasm, which therefore stains more intensely basophilic than the cytoplasm of less active cells. Inactive fibrous connective tissue cells, or fibrocytes, contain a thin, darkly-staining nucleus and a scanty amount of light blue cytoplasm. A 'fibroblast' is a cell that is actively producing collagen fibers and matrix material; since it is a protein-producing cell, it needs the RNA that makes its cytoplasm basophilic, and it therefore must have visible and sometimes multiple nucleoli in the nucleus. The term 'fibrocyte' refers to cells which have passed into a quiescent phase, in which they are not concerned with production of collagen, just maintenance of the fibers that have already been produced. In wound repair and normal growth, active fibroblasts are quite common, but their proportion of the total connective tissue cell population is reduced when there is no new fibrous tissue being made. However, the distinction between '-blast' and '-cyte' is largely academic, since the change from one state to another is reversible. The matrix within which the connective tissue cells reside is composed of glycoproteins and glycosaminoglycans that contain intermingled fibers (collagen, elastin). Collagen is coarsely fibrillar and may be aligned in straight, parallel bundles, with the fibroblasts and fibrocytes scattered throughout the abundant tissue matrix. It is not uncommon to find rare well-granulated mast cells in connective tissue; on histopathologic samples, these are especially common around blood vessels.

**Capillary Endothelium** - Endothelial cells form a monolayer lining the surfaces of blood vessels and lymphatics, and as such are found in all tissues. Fragments of capillaries are only rarely found in aspirates, but are often seen when corings, imprints, or scrapings of well-vascularized tissues are examined. Although individual endothelial cells may be present on such smears, it is probable that they are generally mistaken for fibrocytes (or ignored completely), since both cell types have spindle-shaped nuclei. Endothelial cells are often seen as bare, thin, cigar-shaped nuclei without cytoplasm, since the cytoplasm of the capillary is frequently disrupted during the collection process. Capillaries appear as chains of these individual cells. Branching of capillaries is commonly seen, and tangles of whole capillary nets may be exfoliated from fragile tissue. It is common for a few erythrocytes to be seen within capillaries, which helps in the identification of the structures as blood vessels. The RBCs will be distorted and elongated as they squeeze through the lumen of the vessel. Arterioles and venules are much thicker than capillaries, and arterioles demonstrate circumferentially arranged smooth muscle nuclei as well as longitudinally arranged nuclei, giving them a 'fuzzy caterpillar' appearance.

**Adipose Tissue** - Fat cells, or adipocytes, are very large and spherical, ranging up to about 130 microns in size. The cells have a signet-ring appearance, with the bulk of the cytoplasm occupied by a single large fat droplet rimmed by a thin margin of cytoplasm. The single nucleus is ovoid in appearance, and is usually displaced to one side of the cell, presumably pushed aside by the fat droplet in the cytoplasm. It is unusual to find intact adipocytes in cytologic aspirates, since the cells rupture during the aspiration procedure; instead, one finds fat droplets of varying sizes. In coring preparations or scrapings, adipose tissue may be found as single cells, groups of cells, or extensive masses of cells interspersed with capillaries and stromal elements (small spindle cells representing the fibrocytes of fibrous connective tissue). The groups of adipocytes have the classical 'chicken wire' appearance. In aspirates, non-staining fat droplets may be found in the background from ruptured lipocytes, since most lipocytes do not survive the aspiration process intact; only a few scattered cell membranes ('ruptured balloons') from the cell remnants remain. This is why corings of suspected lipomas are preferred over aspirates; the cells in corings of these fatty tumors are preserved and are not ruptured, and thus the cell type can be ascertained.

**Muscle** - The striated (skeletal) muscle cell is an elongated cell which is multinucleated and tapered or blunt on the ends. Fragments of muscle tissue may occasionally be found on corings or scrapings as deeply basophilic-staining rectangular blocks of cells with ovoid to fusiform nuclei located along the lengths of the muscle fibers. Cross striations are easily detectable within the cytoplasm as thin dark bands oriented perpendicularly to the long axis of the cells; these are best visualized when focusing up and down on the slide.

Other cell types which may be found in variable numbers in cytologic preparations of normal tissue include:

**1) Tissue histiocytes or macrophages** - The tissue histiocyte is an extremely pleomorphic cell which may be found in cytologic preparations from any organ, and these are common in preparations from dermatologic conditions. The histiocyte has a round to ovoid nucleus and a moderate amount of lightly basophilic cytoplasm that may contain a few vacuoles. Phagocytic histiocytes (macrophages) are larger cells that may be multinucleated and have prominent nucleoli. They have a large amount of cytoplasm more or less filled with phagosomes containing ingested material. Epithelioid histiocytes are seen in granulomatous processes; these cells are the Langerhans or antigen-presenting cells. These poorly-phagocytic histiocytic cells have slightly to moderately elongated nuclei and abundant cytoplasm, as compared to the round nuclei of conventional histiocytes; some show 'footprint'-shaped nuclei. Antigen-presenting cells do not generally show phagocytosis; their job is 'nibbling' and antigen presentation to the T lymphocytes. For this reason, they are easily confused with either epithelial cells or sarcoma cells.

**2) Mast cells** - Mast cells are occasionally found in most cytologic preparations that include dermis and subcutaneous tissue, and are especially common in samples obtained from the skin. It is important to note that the finding of a solitary mast cell is unlikely to have any pathologic significance, especially since these cells are common surrounding blood vessels in the fat. On the other hand, since it is impossible (without molecular methods) to distinguish a normal mast cell from a tumor cell obtained from a well-differentiated mastocytoma, the finding of more than a few mast cells in a cytologic specimen warrants consideration of neoplasia. Mast cells are commonly seen in feline lymph nodes, in the skin of dogs that commonly have atopy (especially the Shar Pei) along with eosinophils, and in other specific conditions in which there are many eosinophils (such as feline eosinophilic granuloma/plaque).

Normal mast cells are round and contain many dark purple granules. Intact cells may contain so many granules that the round, centrally-located nucleus is completely hidden. The purple color of mast cell granules may leach out during staining, leaving the cell with a centrally located round nucleus and pale cytoplasm surrounded by a deep magenta 'halo'. This is common in feline mast cells found in various tissues (especially intestine), and may make it difficult to identify them as mast cells. Alternatively, in heavily granulated mast cells, the granules may take up so much stain that the centrally-located nucleus does not stain at all. Since mast cells are somewhat fragile, it is common for them to rupture during slide preparation, releasing their granules into the background.

Mast cells are derived from a hematopoietic precursor cell in the bone marrow; they normally mature in connective tissue. Thus, it would not be surprising to find the odd mast cell in scrapings or corings of subcutaneous tissue such as fat or muscle. It is not uncommon to see mast cells in certain soft-tissue sarcomas, especially those that are well vascularized. In some of these tumors, mast cells can be found in confusingly large numbers scattered among the sarcoma cells. In some aspirates from soft-tissue sarcomas, the mast cells exfoliate well while the sarcoma cells do not---leading to a misdiagnosis of mast cell tumor. The reason for the large number of mast cells in some of these sarcomas and not in others is unknown.

**3) Plasma cells** - Another cell that may be found in the dermis (and which is increased in numbers in chronic inflammation) is the plasma cell. Plasma cells have a nucleus with areas of condensed chromatin; the nucleus is the same size as a small lymphocyte, but there is a great deal more cytoplasm. Most are 14-20 microns in diameter. The nucleus is generally round and eccentrically located and is small in contrast to the volume of cytoplasm, the nuclear chromatin is condensed (but we do not see the 'clock face' in cytology samples that is typical of plasma

cells in tissue biopsies processed for histology), and the cytoplasm is quite basophilic. Although most plasma cells have a single nucleus, it is not uncommon to see rare binucleated plasma cells in conditions in which there is proliferation of plasma cells.

Nuclei generally take up basic stains, i.e., those which are alkaline, because they contain comparatively large amounts of acidic material (deoxyribonucleic acid (DNA) and ribonucleic acid (RNA)) which has a strong affinity for alkaline stains. This is called basophilia or a basophilic reaction. Cytoplasm tends to be poorer in acidic materials than the nucleus, causing the cytoplasm to stain palely or very lightly basophilic. However, when cells synthesize a great deal of protein (and therefore contain much cytoplasmic RNA), the cytoplasm may be quite basophilic. This is the case with plasma cells, which actively produce antibodies. Frequently, in plasma cells and in other cells with abundant, basophilic cytoplasm, one will see a visible 'Golgi zone'. This is a cellular organelle named for its discoverer, Camillo Golgi. The Golgi apparatus is intimately involved with the chemical modification of proteins synthesized in the rough endoplasmic reticulum, frequently by adding sugars to these proteins. It also helps to package these protein/sugar complexes into membrane-bound vesicles for transport to the surface of the cell and release. The Golgi apparatus is found in almost all cell types, but it is usually quite inconspicuous. It is located in the perinuclear area and is very pale, since it has no ribosomal RNA to cause affinity for the basic stains. It is therefore invisible in cells that have pale cytoplasm, but is seen in all cells that have very basophilic cytoplasm. The Golgi apparatus is especially visible in cells that actively make a product and secrete these products rapidly, which is why we can see a perinuclear 'Golgi zone' in most plasma cells.

Sometimes we see plasma cells that make 'packaged' antibodies, which may fill the cytoplasm with round, pale to light blue inclusions. These are called Russell bodies, and the plasma cell that is filled with these is called a Mott cell. These cells are plasma cells that produce large amounts of immunoglobulin contained mainly in large vesicles, the so-called Russell bodies. When viewed under the light microscope, Mott cells often appear to consist solely of an aggregation of Russell bodies. Mott cells are thought to represent a pathological state of plasma cells, the final stage of B-lymphocyte development, when immunoglobulin is secreted at a high rate. If, for any reason, plasma cells produce immunoglobulin at a rate significantly faster than it can be secreted, it will accumulate in the lumen of the endoplasmic reticulum to form large vesicles, which give the Mott cell its phenotypic appearance. We do not know why these particular plasma cells do not release their proteins and instead package them, but some cytologists jokingly call them 'constipated' plasma cells. It is important to be able to recognize this plasma cell variant since otherwise one might think that it was a cell filled with some kind of organism!

Occasionally one finds plasma cells that make brightly eosinophilic antibodies that fill the cell with magenta material; these are called 'flaming' plasma cells. The 'flame' appearance of the plasma cells, with the red color to the antibody material the plasma cells are producing, is interesting. These picturesque cells are characterized by fiery fringes, which are formed by pseudopodic cytoplasmic projections that stain bright pink to carmine red with Wright-Giemsa-type stains. The devitalized peripheral cytoplasm contains numerous dilated endoplasmic reticulum cisterns, which are distended with immunoglobulin. The secretory obstruction destroys and thickens the cell margins, eventually causing the dying cell to shed these hardened fragments of immunoglobulin-laden cytoplasm. This process is known as clasmatosis. Fragments of red cytoplasm may be seen scattered about the smear. Flaming plasma cells have classically been associated with immunoglobulin A (IgA) myelomas; their characteristic staining is thought to be related to the high carbohydrate content of IgA molecules. However, flaming plasma cells have also been seen in association with other globulin-secreting types of myelomas, as well as in reactive plasmacytosis.

**4) Melanocytes** - Cells that synthesize melanin and distribute it to epithelial cells and hair are called melanocytes. It is not uncommon to find a sprinkling of brown melanin granules within epithelial cells from the skin, and some adnexal tumors contain epithelial cells that are melanized as well. Melanocytes are occasionally found in cytologic preparations from the skin or conjunctiva of domestic animals. They are usually round to ovoid and contain abundant melanin granules that are uniform in size and are black or brown in color. The nucleus is round and may be obscured by the large number of granules. Melanocytes often rupture during aspiration or slide preparation, releasing their granules into the background.

5) **Wandering cells from the blood**, such as neutrophils, lymphocytes, and eosinophils, may also be found on cytologic preparations collected from any tissue, and are especially common in chronic dermatologic diseases. There are generally easily recognized, since they are ubiquitous throughout the body.

Once one has the ability to recognize the 'normal' cells from the skin, one can move on to study the neoplasms that arise from these cells.

## NOTES

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

# CYTOLOGY OF CUTANEOUS NEOPLASIA: CLASSIFYING TUMORS AND DETERMINING CYTOLOGIC CHARACTERISTICS OF MALIGNANCY

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Cells from 'normal' tissues live harmoniously, maintaining an appropriate distance from each other without 'piling up'. They divide only at the rate necessary for replacement of tissue which has died from aging or injury. Normal cells from the same organ are also homogeneous and predictable---each cell looks much like the next cell. The nuclear/cytoplasmic (N:C) ratio should range from 1/3 to 1/10, depending on the tissue type. An exception to this is the lymphocyte, which normally has an N:C ratio of 1/1. Nuclei and nucleoli should generally be uniform in number per cell, size, and shape---if nucleoli are even large enough to be visible. Mitotic figures are encountered only rarely or not at all in most tissues. In fact, totally normal cells from the same organ are striking in their 'sameness'.

Normal cells react to their environment and to many pathologic processes by degenerating, repairing, and by reacting to chronic irritants. These processes lead to *dysplasia*, in which certain nuclear and cytoplasmic characteristics can resemble neoplasia. It is usually possible to differentiate neoplastic from dysplastic processes in cells exfoliated from a solid tissue, since one has a clinical history, radiographs, and the presence of a mass to suggest that a neoplasm is present. In dysplasia, there is often a history of a chronic irritant or infection, and the presence of inflammatory cells along with the suspicious cell population makes one unwilling to diagnose a tumor without a histopathologic biopsy.

Although tumors with marked cellular atypia are not uncommon, a large proportion of neoplasms are of low-grade cytologic malignancy. The cytologist may be hard-pressed to make a definitive diagnosis---benign versus malignant---in these well-differentiated tumors. It is a working principle of cytologic diagnosis that a tumor with many characteristics of malignancy may be interpreted as 'malignant'. On the other hand, aspirates which have few malignant characteristics may *not* be interpreted as 'benign'. A carcinoma of low-grade malignancy may have cells which appear cytologically normal, but the cellular architecture of the tissue may be disarranged. On histopathologic examination, the tumor may show invasion into the subcutaneous tissue, 'piling up', and other structural abnormalities not noted on a cytologic preparation. 'Benign' is a histopathologic diagnosis only! For these reasons, an epithelial or glandular nodule *without* characteristics of malignancy should be interpreted, for example, as an 'adenoma/well-differentiated adenocarcinoma'. A melanocytic tumor *without* appropriate numbers of characteristics of malignancy should be interpreted as a 'melanocytoma or well-differentiated melanosarcoma'.

Keeping in mind these cautionary statements, the cytologic characteristics denoting malignancy may be examined. Malignant cell cytology demonstrates characteristics typical of anaplasia or de-differentiation. Unfortunately, there is no one criterion which, when present, unequivocally signals cancer. There are, however, 'strong' characteristics which more reliably suggest malignancy; most of these strong indicators are found in the nucleus. Several 'weak' criteria---usually cytoplasmic---can be used to support a diagnosis of malignancy but cannot by themselves make the determination.

The morphology of the nucleus is of the utmost importance in cytologic evaluation, since it reflects the biologic activity of the cell. **Enlargement of the nucleus** is often found in cancer, and cells with nuclei larger than 10 microns in diameter are usually malignant. This nuclear enlargement is due to polyploidy, or doubling of DNA without subsequent nuclear division. In some particularly anaplastic tumors, dramatic disparity in nuclear size from cell to cell may be seen. With variation in nuclear size comes an **increase in the N:C ratio, sometimes to 1:1**. In general, the degree of cellular maturity is inversely proportional to the N:C ratio, i.e., the more immature the cell the higher the N:C ratio. To properly assess N:C ratio, however, the cytologist must be familiar with the normal range of N:C ratios for that cell type. For example, immature transitional cells obtained with a biopsy instrument from the

underlying bladder epithelium have a much higher N:C ratio than do surface transitional cells. Slight variation in nuclear size and mild alteration of the N:C ratio may be seen in dysplasia.

**Irregularity of the nuclear membrane** is an abnormality which is generally not seen in benign or dysplastic cells, and as such is a strong indicator of malignancy. These irregularities may appear as pointed spicules, sharp infoldings or outward projections, or nuclear 'buds'. **Multinucleation as the result of abnormal mitosis** is sometimes seen in neoplasia. It must be remembered, however, that multinucleation occurs also in benign cell types such as macrophages, giant cells, mesothelial cells, plasma cells, osteoclasts, spermatocytes, hepatocytes, and transitional cells. In general, nuclei of benign multinucleated cells are isokaryotic; multinucleated tumor cells often show mild (and sometimes marked) anisokaryosis. As has been previously mentioned, mitoses are infrequently seen in normal tissues, the exceptions being bone marrow and mesothelium. Normal mitoses do not help to definitively diagnose cancer, since they are commonly found in dysplasia. However, **abnormal mitotic figures or 'stray' or 'orphan' chromosomes** left over after mitosis has been completed strongly suggest malignancy.

**An increase in size and number of nucleoli** may be seen in cells that are actively synthesizing protein. Therefore, prominent or numerous nucleoli only reflect a heightened level of cell protein synthetic activity, which may be seen in dysplasia or neoplasia. However, **disparity in nucleolar size and shape within the same cell, or variation in the number of nucleoli in cells within the same tissue fragment** strongly suggests malignancy. **Increased basophilia of the cytoplasm** implies increased RNA content of the cell and suggests rapid cell growth. Some cells may have such blue cytoplasm that visualization of the nucleus within the cytoplasm is not possible. This cytoplasmic hyperchromasia is a weak criterion of malignancy, since it may be seen in any tissue undergoing rapid growth.

How many of these characteristics should be found to be sure that a tumor is malignant? The answer depends partially on whether the characteristics are *strong* or *weak* indicators of malignancy. The characteristics of extreme nuclear or nucleolar variation in size, abnormal mitosis, indentation of the nuclear membrane, variation in nucleolar shape, and multinucleation with anisokaryosis provide strong evidence for malignancy. Other characteristics, such as basophilic cytoplasm, mild to moderate nuclear size variation, normal mitotic figures, variation in nuclear number per cell, and multinucleation with isokaryosis are weaker indicators. Luckily, many aspirates contain enough strong malignant characteristics that the diagnosis is straightforward. However, the finding of even a few cells with *strong* malignant characteristics should make the cytologist strongly suspicious that the mass is malignant. The presence of abnormal cells with weak characteristics *may* mean malignancy; on the other hand, it may indicate only a reactive or dysplastic process. This is especially true if chronic infection or inflammation is superimposed on normal tissue, as in pyoderma or granulomatous inflammation. Dysplastic cells may show deep blue cytoplasm, multinucleation (with isokaryosis), an increased N:C ratio, and variable nucleolar numbers. The cytologist should equivocate ("I do not know, but I do not trust it.") and request a follow-up cytologic examination after the infection has been controlled. The continual finding of abnormal cells warrants a surgical biopsy.

## **CLASSIFYING TUMORS AS EPITHELIAL, MESENCHYMAL, OR ROUND-CELL**

In order to differentiate epithelial, mesenchymal, and round cell tumors, the cytologist must evaluate individual cell morphology and patterns of cell arrangement. As a group, the epithelial tumors exfoliate well, and the cells are often found in grape-like clusters. Epithelial cells tend to be cohesive because of the presence of desmosomes ('intercellular bridges'), and lines of cell-to-cell adherence may be found within the clusters. Individually, the tumor cells are typically round with well-defined cell membranes. When there is evidence of secretory activity in a malignant tumor, with secretory cytoplasmic droplets or signet rings, a diagnosis of adenocarcinoma can be made.



Adnexal structures of the skin are common sites of neoplasia in the canine species and are also seen in cats. These tumors often have large numbers of basal cells within them; these have a grape cluster-like appearance. Unless one can see evidence of differentiation, one cannot tell which adnexal structure they are arising from.

Older dogs often have large numbers of superficial wart-like growths which are identified by histopathology as hyperplastic or adenomatous sebaceous glands. Hyperplasia and neoplasia of sweat glands also occur in canine skin, but with less frequency. In adenomatous hyperplasia and adenomas of sebaceous glands, most of the cells are mature secretory cells. The centrally-located nuclei are usually completely obscured by droplets of secretory material. In compound adenomas and epitheliomas, however, many basilar 'reserve' cells are commonly found; these are immature cells containing little or no secretory material. Since the cytoplasm of reserve cells is basophilic and the N:C ratio is approximately 1:2, the cytologist may incorrectly diagnose these tumors as malignant.

Benign (apocrine) sweat gland and ceruminous gland tumors are composed of cuboidal to low columnar cells which sometimes contain basophilic cytoplasmic inclusions. It is not possible to reliably distinguish these tumor types by cytologic examination. Ceruminous gland masses are particularly difficult to define as malignant, since there is often concurrent severe inflammation, infection, and resulting dysplasia in the tissue within the ear canal. Thus, histopathologic evidence of invasion and the finding of increased numbers of mitotic figures are necessary to make a determination as to whether the lesion is dysplastic or neoplastic.

Aspirates from sebaceous or sweat gland adenocarcinomas consist of clusters of highly basophilic reserve cells with malignant characteristics; only a few cells will contain any secretory material. An occasional cell in an adenocarcinoma will produce a large amount of secretory material; in these cells a 'signet-ring' appearance may be seen, with the nucleus pressed to the margin of the cell membrane.

Cytologically and histopathologically, it is difficult to differentiate hyperplasia of the perianal glands from adenoma. The cells exfoliate individually and in clusters, have uniform nuclei and nucleoli, and contain a large amount of pink-staining cytoplasm which appears grainy at high magnifications. The cells so closely resemble liver cells that European literature has termed perianal gland neoplasms 'hepatoid gland tumors'. In some aspirates, a row of flattened 'reserve' cells may be seen surrounding the larger cells. The cytoplasm of these small cells is more basophilic and the N:C ratio ranges from 1:1 to 1:2. These cells differentiate into the large hepatoid cells. The finding of large numbers of reserve cells on a cytologic aspirate from a perianal gland tumor rather than the normal single layer around the hepatoid cells is very worrisome for malignancy.

Squamous cell carcinoma is a tumor that originates in the surface epithelial layer of the skin; it is commonly found in all domestic species. The cell of origin is the keratinocyte, and the tumor characteristically produces keratin. Since keratinizing cells lose their tendency to cluster and usually exfoliate as individual cells, the inexperienced cytologist may have difficulty even identifying these tumor cells as epithelial in origin. Normal keratinocytes have a low N:C ratio ranging from 1:8 to 1:10. As a squamous cell keratinizes, the nucleus dies, undergoing pyknosis, karyorrhexis, and finally disappearing altogether, leaving an anuclear squame. A well-differentiated squamous cell carcinoma with little cellular variation from normal may be difficult to distinguish from dysplastic conditions of the skin resulting from chronic irritation or infection. Tumor cells from these well-differentiated carcinomas show so little atypia that they might even be considered normal in another context. Isolated keratinizing squamous cells exfoliate easily from the tumor, and are large with basophilic cytoplasm. In the same smear, however, there are usually cells which are multinucleated or have large nuclei undergoing pyknosis. These abnormal cells may have N:C ratios from 1:3 to 1:5, which would not be considered abnormal for epithelial cells from other organs. The large nuclei frequently show incipient karyorrhexis, a cytologic change characterized by vacuolization of the dying nucleus. Degree of differentiation of a squamous cell carcinoma is assessed by the degree of cellular anaplasia as well as the degree of keratinization. In some tumors, anaplastic cells with giant and polymorphous nuclei are found interspersed among small keratinized squamous cells and keratin debris. In poorly-differentiated squamous cell carcinomas, the cells exfoliate in the

clusters typical of other epithelial neoplasms. Individual keratinizing cells are usually also found on the smear, allowing the cytologist to make the diagnosis of squamous cell carcinoma.

It is typical of cells from tumors of mesenchymal origin to exfoliate poorly as compared to epithelial tumors and round cell tumors. Despite vigorous aspiration, the cytologist may obtain only a drop or two of blood and a few scattered cells; imprints of a biopsy sample may be equally unrewarding. Generally, a scraping from a biopsy sample will provide the most cytologic material for interpretation. Rather than exfoliating in clusters, cells from mesenchymal tumors are found lying separately, in individual units. A vigorous scraping may result in thick sheets of cells on the slide, which can confuse the inexperienced cytologist into believing that the cells are 'clustering'. However, examination of an area in which the cells are spread thinly will reveal that the cells show no evidence of cell-to-cell attachment, unlike the epithelial tumors. Typically, the cells of benign and malignant mesenchymal tumors have poorly-defined cell membranes as compared to epithelial tumors. The cells are not generally round; they may be spindle-shaped, polygonal, or dendritic, but uniform 'roundness' is a characteristic of epithelial cells or of cells from a round cell tumor. In the skin, the most common tumors of mesenchymal origin are the spindle-cell tumors. The benign and malignant spindle-cell tumors comprise a group of mesenchymal tumors which are difficult to distinguish cytologically: the fibroma/fibrosarcoma, myxoma/myxosarcoma, and the neurofibroma/neurofibrosarcoma. Hemangiopericytoma is a spindle-cell tumor arising from the perivascular wall in the subcutaneous tissue of the skin. This tumor is often found on the limbs of older dogs; it is possible to diagnose cytologically by its uniformly round nuclei and wispy, diaphanous cytoplasm.

Exfoliation of cells from the spindle-cell tumors is typically extremely poor, but a scraping will provide adequate cells for examination. Cytologically, cells from benign or well-differentiated malignant spindle-cell tumors will have fusiform or ovoid nuclei with little or no cellular atypia. A word of caution should be inserted here: Actively proliferating granulation tissue will contain many young, rapidly-dividing fibroblasts, and these may be mistaken for neoplastic cells. Poorly-differentiated malignant spindle-cell tumors have nuclei which vary from a homogeneous fusiform shape to pleomorphic round or oval forms. Characteristics of malignancy may be marked, including cytoplasmic basophilia of individual cells, mitotic figures, and marked nuclear/nucleolar variation in size and number. An increased number of mitotic figures per field has been shown to portend a poor prognosis in both canine and feline fibrosarcoma, and the mitotic index is prognostic for most soft-tissue sarcomas. In some spindle-cell tumors, the cells are so anaplastic that no 'spindling' cytoplasmic extensions are present, and the cytologist must call the tumor an 'anaplastic sarcoma'.

Dermal melanin-producing tumors are common in the dog and horse, but are found only rarely in the cat. Although most cutaneous melanomas are benign, canine tumors found on the lips, digits, and in the oral cavity have a high incidence of malignancy. While aspirates of many other non-epithelial tumors are cell poor, a moderate to large number of cells is generally obtained from dermal melanin-producing tumors. Occasionally, only scattered cells mixed with blood are aspirated, but melanin pigment will almost always be found in the background. In extremely melanotic tumors, the aspirated material and the unstained smear may contain so much pigment that they are colored brown-black. The presence of melanin pigment staining black or brown with Romanowsky stains is suggestive of the diagnosis, since the melanoma cells often rupture during the aspiration procedure. It must be remembered, however, that pigment per se is not diagnostic of a melanin-producing tumor, since dark pigment is present in other benign cell types. Hemosiderin in macrophages, keratohyalin granules in squamous epithelial cells, and the occasional normal melanocyte found in skin may lead to an erroneous diagnosis. Tumors arising from the Meibomian gland sebaceous cells in dogs are often melanized. Macrophages (melanophages) commonly phagocytize free pigment from lysed cells and may also be confused with tumor cells. Melanophages tend to be vacuolated and larger than the tumor cells, however. Phagocytized melanin pigment is coarser and in variably-sized 'packets', as compared to the finer, uniform granulation typically found in melanoma cells. Pigment in the melanin-producing tumor cells may be absent (amelanotic melanoma), appear as a salt-and-pepper sprinkling in occasional cells, or may fill the cell with black masses, sometimes nearly obscuring the nucleus.

Because of the embryonic neuroectodermal origin of the melanocyte, cell distribution is sometimes confusing. The tumor may appear in areas to be mesenchymal and in other areas to be epithelial. Although aspirated tumor cells are usually dissociated, sheets of cells and rare clusters may be found. Cell morphology varies from round to ovoid to spindle-shaped. Malignant melanomas in which the cells are generally round are typed as epithelioid melanomas, while tumors composed principally of fusiform or stellate cells are typed as spindle-cell or dendritic melanomas. Animals with purely epithelioid tumors have been reported to have a shorter survival time than those with spindle-cell, dendritic, or mixed tumors. Cytoplasm in most melanoma cells is abundant, and the N:C ratio may be low even in highly malignant tumors. Few cytologic characteristics of malignancy are found in well-differentiated melanomas. In general, the less pigment present in the tumor, the more anaplastic its cellular characteristics. Anisokaryosis and many mitotic figures are usually found in poorly melanotic tumors. The degree of tumor pigmentation has not been reported to consistently predict prognosis, but animals whose tumors contain large numbers of mitotic figures have a comparatively short survival time. Ultimately, the differentiation as to benign or malignant in a melanocytic tumor is made histopathologically with the mitotic index. Diagnosis of amelanotic melanomas is difficult for both the cytologist and the histopathologist, and immunohistochemistry (Melan-A staining) may be required if a definitive answer is needed. It is this author's experience, however, that the cytologist conducting a careful search can usually find an occasional cell with fine pigment even in poorly melanotic tumors; such fine cellular details may be missed by the histopathologist.

The round-cell tumors as classically defined consist of transmissible venereal tumors, mast cell tumors, lymphomas, histiocytomas, and plasma cell tumors. However, other tumors may also fit morphologically into this 'round cell' classification---the other histiocytic neoplasms, some liposarcomas, extraskeletal osteosarcomas, some amelanotic melanomas, and anaplastic carcinomas (which have lost their characteristic clustering). The round-cell tumors are separated as a class from the epithelial and mesenchymal tumors because of cytologic characteristics which set them apart. Typically, the round-cell tumors exfoliate well, and cytologic preparations may easily be too thick to interpret except at the edges of the aspirate. The cells are round---like epithelial cells--and have distinct cell membranes. Like mesenchymal tumors, however, the cells from a round-cell tumor exfoliate individually rather than in clusters. One has the overall impression of many homogeneous round cells with no cell-to-cell attachment. As a rule, the cytologic characteristics used to judge malignancy in epithelial and mesenchymal tumors are not applicable for the round-cell tumors, since the cells may be totally uniform and yet behave malignantly.

## NOTES

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# **PARASITICIDES**

Dr. John MacDonald

Not available at print time.

# PERMETHRIN INTOXICATION OF CATS

Malik R<sup>1</sup>, Page SW<sup>2</sup>

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The pyrethrum pesticides were originally derived from extracts of *Chrysanthemum* (Tanacetum) flower heads. Synthetic derivatives, known as synthetic pyrethroids or SPs, were developed to improve and broaden pesticidal efficacy and photostability. The most commonly used member of this class of drug currently used in companion animals is permethrin, a type 1 SP. Permethrin acts to modify the kinetics of the voltage dependent sodium channels, resulting in depolarisation and repetitive firing of neurones in the central nervous system, especially in the spinal cord.

**While SPs are generally considered very safe to mammals, the cat is extraordinarily susceptible, for reasons that have not been elucidated.** Delayed rate of metabolism (which involves both esterases and glucuronidation) would appear to be only a component of the problem. Permethrin is widely regarded as the most common cause of intoxication in pet cats in both the United Kingdom and the USA. **The hallmark of permethrin toxicity is muscle fasciculation and whole body tremor. The tremor is typically coarse, and may subsequently progress to generalised motor seizures, and then coma and death.** Signs of sympathetic overactivity and hyperthermia can be present also. **An important historical clue to diagnosis is application of an over-the-counter (OTC) permethrin “spot on” (PSO) product designed for use on dogs, close contact with a treated dog, or even contact with an area sprayed with high concentrations of permethrin or related SPs (e.g. when the environment is treated for fleas or spiders).** Curiously, little is known about the toxicity of permethrin in cats but a lethal dermal dose of 100mg/kg has been described in a case report; at this dose rate, **each of the dog PSO products available in Australia contains at least one (and up to eight) lethal dose(s) for a 4 kg cat.**

One article in the *Australian Veterinary Practitioner* suggested no cases of SP intoxication in cats in Australia were recorded from January 1995 to May 2003. Two articles in more recent issues of the *Australian Veterinary Journal* (AVJ) have described cases of permethrin toxicosis in cats following inappropriate treatment with high concentration PSO products labelled for use in dogs only and bearing the constraint statement: “Do not use on cats”. Dymond & Swift (2008) presented a retrospective study of 20 cats treated at an emergency clinic in Brisbane from October 2004 to June 2005. This latter case series had been presented in preliminary form at a conference (Science Week of the Australian College of Veterinary Scientists) in July 2005. An emotive letter to the editor of the AVJ by the first author and feedback from the senior author in his capacity as chair of the **Therapeutics Advisory Committee** of the *Australian Veterinary Association* (AVA) provided the impetus to obtain a more quantitative insight into the extent and nature of the problem, and to dissect out factors that may contribute to increasing incidence of this intoxication in cats.

A survey was therefore conducted by the **Centre of Veterinary Education** (CVE) of the University of Sydney working in concert with the **Australian Small Animal Veterinary Association** (ASAVA). A total of 207 practitioners and/or clinics reported having attended to cases of permethrin intoxication in cats over the last 2 years. **A total of 750 individual cases were reported with 166 deaths.** Although death was generally attributable to intoxication *per se*, in 39 cases affected cats were euthanased at the request of owners unable to pay the anticipated costs of hospitalisation and therapy. The survey identified Exelpet Flea and Tick Eliminator (Mars Australia)<sup>TM</sup> (Figure 1), Bayer Advantix<sup>TM</sup>, and Purina Totalcare Flea Eliminator Line-On<sup>TM</sup> as the brands most commonly implicated, although it was not possible to identify the specific product applied from the practice records in many instances. Permethrin-containing formulations were most commonly obtained from supermarkets, followed by pet stores, veterinary clinics, and a range of other outlets including produce stores and friends. Except for six patients, cases of intoxication of cats by permethrin involved the use of “spot-on” products labelled only for use in dogs with specific label instructions e.g. “DO NOT USE IN CATS”. Typically,

intoxications resulted from (i) failure to notice the warning icon (ii) untrained staff giving instructions to ignore the warning label (iii) the owners decision to use the product despite the warning, often at a reduced dose (iv) administering the product in error (v) being given the product by a friend who owned a dog (vi) secondary contact e.g. contact with a dog or brush treated with permethrin.



**Figure 1. Note that the warning icon (arrow) on the cardboard box is small. Further warnings do not appear on either the foil packet or the applicator pipette (thicker arrow) – the latter having no labelling what so ever.**

The commonest reasons that cats are subject to permethrin intoxication, based on the comments of the respondents are summarised below. Many of these comments were provided independently by several respondents, with minor variations on the theme. Hopefully the sense of these numerous anecdotes has been captured, in point form below:

1. Owners do not invariably notice or take heed of the warning icon on the product label. This is especially an issue for older owners with poor vision or when English is not the owner's first language. Many owners think/claimed they are applying Frontline™ irrespective of what product they were applying. Similar statements from owners are reported by Meyer (1999) from a study in the US.
2. Many owners do not understand that “toxic to cats” or “do not use on cats” means that the cat can actually die as a result of application of the product.
3. Some owners believed the warning label is spurious and present only to encourage them to buy more expensive feline preparations.
4. Owners sometimes deliberately buy a large canine applicator to treat many cats, or a dog and several cats – resulting in multiple intoxications. And often multiple fatalities.
5. Owners are sometimes given incorrect advice at pet stores, supermarket and produce stores that the products are indeed safe for cats, despite labelling to the contrary.
6. Certain pet stores open the containers and make the product available without the immediate or outer warning label being apparent. This is not legal. But it is apparently a widespread occurrence.
7. Some people get cat and dog doses out at the same time – but then use the incorrect applicator i.e. give

the cat the dog dose.

8. People may be given products from friends – but applicators, having been removed from their packaging, may no longer have a warning label. This is especially an issue for the Exelpet range – as the warning label is only present on the outermost cardboard container – and the individual applicator pipettes have no writing on them whatsoever, and no warnings. This can also happen within a household if the external cardboard container is not used to store unused applicators. It must be emphasised, however, that even with exemplary labelling, as in the Advantix product where each layering of the product has a warning of some sort, feline intoxications continue to occur because people fail to read or appreciate the significance of warning symbols.
9. Some people use a tiny dose, having already treated their dog or dogs – they think a small dose (e.g. one drop) will be safe for the cat, but it proves sufficient to cause severe signs.
10. One owner applied some to a cat because it was annoying him while he was treating his dog. He knew he was being “naughty”.
11. Many owners do not appreciate that close contact with a dog or brush may be enough to intoxicate the cat in the first 24 hours after application of the product. One cat licked the product off its companion dog and became intoxicated, while several in a household were poisoned by being combed after the owner had first used the comb on the family’s dog, which had had a permethrin product recently applied.
12. Some colleagues are concerned about the potential for malicious use of products that are so freely available.

Finally, we quote one respondent who captured the sense of what many of her colleagues had on their mind **“It is one of the most frustrating things in practice when we see these cats. The owners are always very ashamed, and regretful, and most of them were trying to save money, and didn't really read the label. And since they were trying to save money in the first place, they then can't or won't justify the cost to treat the cat. They have no idea cats are more sensitive to certain things than dogs. I think it is very irresponsible of supermarkets to sell products that can very easily be lethal to animals.”**

Over the last ten years there have been many letters, case reports and small case series in the UK and North American veterinary journals and magazines from individuals, veterinary emergency centres, and poison information centres concerning the dangers of pet owners mistakenly using canine PSO on cats. It is well accepted that the dog dose provided in the applicator tubes or pipettes contain potentially lethal doses for cat(s) and that many deaths have occurred. However, the scale of the problem was unknown until publication of the paper by Nick Sutton and colleagues (2007) which identified 286 new cases from the UK. The same situation appeared to exist in Australia. Several talks at College Science Week from 2005 onwards in the feline, small animal and critical care streams should have alerted the profession to the extent of the problem. Indeed, it was only after publication of the retrospective study by Dymond & Swift in 2008 and the follow up letter by Malik that attention was firmly focused on why permethrin intoxication may have become more prevalent, with considerations such as high interest rates, the GFC and the higher cost of safer “premium” flea and tick products. A bibliography of literature related to the use of permethrin in cats has been published by CVE and is available at <http://www.cve.edu.au/files/Permethrin-Intoxication-of-Cats-Biblio-SWP.pdf>.

The identification of at least 750 cases of feline permethrin intoxication in this survey over a two year period with 166 deaths is a big “wake-up call” to the profession, PSO manufactures, the industry regulator and the public alike. We are reminded of the famous quote sometimes attributed to the English philosopher Edmond Burke: **“The only thing necessary for the triumph of evil is for good men to do nothing”**. We need to emphasise that most veterinarians do not formally report pyrethroid intoxication in cats, either because they are “too busy” or because the use of the drug in question is “off label” on the wrong species and the APVMA reporting system



states that “an adverse experience is an unintended or unexpected effect of a product when used according to the label instructions”. Indeed, it seems likely that our data will have underestimated the number of cases by a substantial margin.

OTC sales of PSO products available from supermarkets, pet stores, pet barns and produce stores account for the majority of cases, although the flea and tick repellent product Advantix™ (available from both veterinarians and pet stores) accounted for a substantial number of cases also. **It is “crystal clear” from the survey that many members of the public cannot grasp the concept that a drug available OTC can be safe for use on dogs but lethal for cats. Furthermore, a small icon comprising a cat with a bar through it and warning such as “Toxic to cats” or “Do not use on cats” provides a patently insufficient warning of the extent of the risk from non-compliance.** Finally, there is insufficient warning that secondary intoxication to cats can result from exposure to dogs treated with permethrin, through contact, mutual grooming or contact with grooming aids.

There is no doubt that PSO products cannot be expected to be used safely without expert consultation and advice. Furthermore, even with the best of veterinary advice, these products pose an unnecessary risk to feline patients because owners are but human and therefore prone to error. **Other far safer and effective products are available for flea control, i.e. formulations that have efficacy not just against adult fleas on the patient, but also on fleas, eggs and larvae present in the patient’s environment,** e.g. as a result of clever drug formulation or the incorporation of insect growth regulators or chitin-synthase inhibitors. Critically, the latest generation of topical and systemic flea treatments (fipronil, imidacloprid, selamectin, methoprene, lufenuron/milbemycin, nitenpyram, spinosad, spinetoram) have very wide therapeutic ratios in cats and dogs, and lend themselves to development of an integrated program for flea control which addresses all stages of the flea cycle. **There is no need for us to tell a group with a special interest in small animal dermatology that utilisation of OTC permethrin-containing products without adequate guidance and counselling is unlikely to provide an effective flea treatment as there is insufficient treatment of the environment.** Furthermore, there is insufficient emphasis on the need for regular treatment and the safe treatment of in contact animals in material available at the point of sale.

**There is a useful literature on the effectiveness of warning labels in this type of setting (see CVE website). Unfortunately, the best available evidence suggests increased labelling and increasing awareness of this problem is unlikely to have sufficient impact on the incidence rate for intoxication, based on experience from the US and UK.** Despite changes to labelling in these countries (principally the addition of a symbol (red circle containing cat with line through) and a statement such as “Do not use on cats” or “Toxic to cats”, permethrin intoxications continue. The number of reported feline exposures to permethrin in the UK increased with publicity and veterinary awareness, confirming the suspicion that cases were being underreported. Fatalities were recorded more frequently post publication, suggesting increased reporting of severe cases. It has been said that “greater similarity between the warning label designer and the intended target group should enhance the effectiveness of the warning label”; this may prove a useful consideration when designing an assessment protocol.

In situations where it is deemed that use of permethrin products in dogs is so compelling that their use is justified, for example in areas with exceedingly high prevalence of the paralysis tick *Ixodes holocyclus* or tick-borne diseases spread by other tick species, then prominent and dramatic use of warning labels (Figure 1) at the time the product is dispensed may offer at least some protection against inadvertent use on feline patients sharing the environment with at risk dogs. Additional and repeated verbal direction from qualified veterinary staff at the point of sale would further reinforce the impact of such labelling.

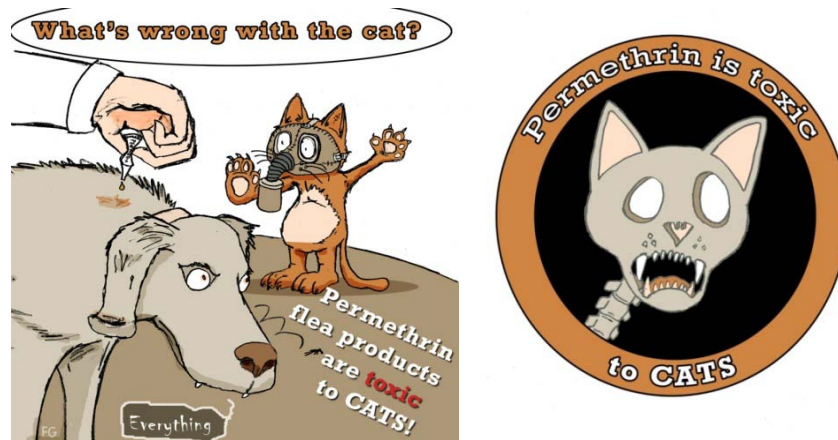


Figure 1. Labels using humour and dramatic emphasis to highlight the extreme risk of PSE products to cats may be utilised as an adjunct to written warnings when veterinarians dispense products such as Advantix to canine patients. These cartoons were created by the veterinarian and cartoonist Frank Gash (Grrinninbear Designs) in support of our campaign.

To sum up, our goal as a profession should be to reduce instances of preventable permethrin intoxication to nil by working in concert with drug manufactures, industry regulators and distributors of OTC PTOs. Our challenge will be to assist regulators in risk communication and risk management, taking into account the results of data in the literature and the most effective ideas and opinions of our profession.

## SUMMARY RECOMMENDATIONS

In the authors' view, because of the likelihood of inappropriate use and toxicity in the non-labelled species, over-the-counter products intended for use in either dogs or cats must have a high margin of safety in both dogs and cats. Furthermore, **PSOs should only be available at points of sale where veterinary advice can be provided and appropriate warnings given. As an interim measure, modified labelling with more explicit warnings may reduce morbidity and mortality.**

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# **CHIARI LIKE MALFORMATION: THE SCRATCHING DOG ONLY A NEUROSURGEON CAN HELP**

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Chiari-like malformation in dogs (CLM) is a condition in which a portion of the cerebellum descends through the foramen magnum, sometimes to the level of the second cervical vertebra, affecting normal cerebral spinal fluid (CSF) flow. The specific cause has not been determined; however, compressive malformations in the caudal fossa, volume mismatches between the cerebellum, brain stem and the caudal fossa, or the loss of the collagenous suspensory mechanism of the cerebellum may play a role in the cerebellar descent. The resultant changes in CSF dynamics cause an abnormal accumulation of fluid within the substance of the spinal cord called a “syrinx” or syringomyelia. It is considered to be a hereditary condition in Cavalier King Charles Spaniels and is commonly confused with many other conditions. Chiari-like malformation in dogs affected approximately 85% of Cavalier King Charles Spaniels evaluated, as reported in the most recent studies.

## **CHIARI-LIKE MALFORMATION IN DOGS**

The name CLM is derived from an analogous condition affecting human patients called Chiari malformation. It consists of a downward displacement of the cerebellar tonsils and the medulla through the foramen magnum and syrinx formation in some patients as a result of abnormal CSF outflow. Austrian pathologist Hans Chiari first described several hindbrain malformations in children in the late 1800's, later named Chiari malformations 1-4. A colleague of Professor Chiari, Dr. Julius Arnold later contributed to the definition of the condition, and students of Dr. Arnold suggested the term Arnold-Chiari malformation to henceforth describe the condition. As the complexity of these conditions is better understood, the number classifications continues to expand including Chiari zero and 1-5, however, the name of the condition generally remains Chiari malformation. Several names have been used to describe the analogous condition in veterinary patients, including Caudal Occipital Malformation Syndrome (COMS) and Chiari malformation in dogs. As a result of a report from the “Chiari-Like Malformation and Syringomyelia Working Group” held at the International Conference on Syringomyelia, at the Royal Veterinary College, London, UK, in 2006, the term Chiari-Like Malformation in dogs was agreed upon to most accurately describe the canine analogue of this condition.

## **SYRINGOMYELIA**

The term syringomyelia (G. *syrinx*, tube + *myelos*, marrow) was first used by Oliviere d'Angers in 1824 describing cystic accumulation of fluid or cavitation within the substance of the spinal cord. Syringomyelia is thought to be a progressive process and a pathologic sequela of altered CSF flow dynamics, mechanical obstruction or both. Hydromyelia describes a dilated central canal with an intact ependymal lining without the extravasation of CSF or the accumulation of extracellular fluid into the substance of the spinal cord (as is seen with syringomyelia). The older term “syringohydromyelia” was sometimes used interchangeably with syringomyelia, but has fallen out of favor to avoid confusing the distinction made between syringomyelia and hydromyelia. The mechanism of the fluid accumulation and the nature of the fluid is

controversial with several theories being reported. Because syrinx formation can occur in any region of the spinal cord, the entire central nervous system is now imaged when patients are evaluated as part of the Chiari screening program at Long Island Veterinary Specialists. In a recent review of nineteen dogs that had complete MRI studies, 19 (100%) had cervical, 9 (47.4%) had cervical / thoracic, 5 (26.3%) had cervical / thoracic / lumbar syrinx formation. These preliminary results suggest further investigation is needed to better understand this complex disease process.

## **CLINICAL SIGNS**

Clinical signs associated with CLM-SM are variable, however results of a large scale ongoing study are found in [Table 1]. The most common and evident clinical sign is pain. Some patients exhibit signs of extreme pain including vocalization from being gently touched or from simply changing position or barking. Pain is most often located in the craniocervical region, however can be located anywhere along the spine and is typically associated with syrinx formation in the region. Signs of scratching are thought to be a form of pain or an attempt to rid the source of pain by some, but for now is regarded as a separate clinical sign. Cervical pain progressing to sensory loss and weakness are attributable to central canal signs and an expanding syrinx. As the hydromyelia breaks through the ependymal lining and fluid dissects and coalesces into the spinal cord, the spinothalamic tracts and the associated dorsal horn grey column are affected first. The result is paraesthesia in the affected dermatome. Typically dogs are affected asymmetrically with correlation to the lateralization of the syrinx. If the syrinx dissection is directed ventrally, weakness, atrophy and lower motor neuron signs become evident first as ventral horn grey matter becomes affected. In severe cases scoliosis is noted as deinnervation of the paraspinal musculature and atrophy occur. Other clinical signs appear more related to intracranial disturbances like seizures, diminished menace response, diminished hearing, and facial paresis.

## **MRI**

The diagnosis of CLM in dogs and Chiari type I in humans can only be confirmed by MRI which is essential for determining the cause of syringomyelia. Descent of the cerebellum or medulla through the foramen magnum, regional CSF attenuation, cerebellar compression are best evaluated on a T2- weighted sagittal craniocervical MRI [Figure 1a&b.)] Objective data is not available regarding normal/abnormal standards when interpreting MR images in veterinary patients and differences between breeds may exist. Because considerable variation exists in the interpretation of images between specialists reviewing MR images, caution is advised when reading interpretive reports. Based on the consensus on interpretation by members of the *Chiari-Like Malformation and Syringomyelia Working Group*, diagnostic standards are likely to be formulated in the near future. Laboratory tests such as hematology, serum chemistry, CSF analysis and urinalysis remain useful in eliminating other possible diagnoses. Advances in MRI technology (CINE-MR) have made possible the analysis of CSF flow which could further our understanding of the effects of CSF flow patterns on syrinx formation. Additionally larger magnets have shortened scan times while enhancing resolution. Because the syrinx can occur from the cervical, thoracic, and lumbar regions, it is important that the brain and entire spine be imaged. Most low cost MRI screen programs will only image the brain and cervical region resulting in need to re MRI dogs needing treatment. The Canine Chiari Institute at Long Island Veterinary Specialists utilizes a 3 Tesla magnet to image the entire nervous system as part of the low cost screening program.

## **MULTI SLICE HELICAL CT / 3D RECONSTRUCTION**

Although encroachment on the cerebellomedullary region by portions of the occipital bone has been the focus of many studies, evaluation of the entire skull shape and size utilizing Spiral CT technology with 3D reconstruction is currently underway to identify additional mechanisms of syrinx formation. Some reports describe a volume mismatch between the caudal fossa volume and its neural contents resulting in compression and herniation as the most common finding. This advanced imaging technique will be able to identify volume or conformational abnormalities in affected patients. Regardless of the cause, the focus is now on correcting the flow of CSF as the malformation affects its normal passage around the brain and spinal cord and leads to the syrinx formation. The results of recent studies at The Canine Chiari Institute at Long Island Veterinary Specialists revealed additional

abnormalities affecting dogs with CLM are frequently found on CT evaluation; now termed craniocervical junctional abnormalities. Proper recognition and treatment plans for these additional abnormalities is essential to long term success.

## **THERMOGRAPHY**

Thermography is a non-invasive imaging technique involving the recording of cutaneous thermal patterns. This imaging modality provides information about the function of the sympathetic nervous system. Because of recent advances in technology and the ability to image patients without the need for sedation, thermography has potential use as a screening test for CLM in dogs. Loughin et al recently concluded a study documenting the reproducibility of image generation in normal canine limbs in 2007. A current study by the same authors is attempting to establish a thermographic imaging protocol for dogs suspected of having CLM. The complete analysis of thermal patterns is on going; however, preliminary results revealed lower temperature thermographic patterns in dogs with abnormal MRI findings compared with the dog with a normal MRI. Magnetic resonance imaging findings classified as mild, moderate and severe correlated with thermographic findings, 100%, 50%, and 0% of the time respectively. Based on these very preliminary findings, thermography may be a viable imaging modality to use as a screening tool to detect CLM in dogs.

## **TREATMENT**

Medical treatment has been directed towards pain relief and the reduction of CSF production. It has had some limited success in improving quality of life, however, no long-term success. The most frequently prescribed medications remain unchanged and include: furosemide (diuretic) and acetazolamide (carbonic anhydrase inhibitor to diminish CSF accumulation), carprofen, deracoxib (non steroidal anti-inflammatory drugs to control pain), prednisone, dexamethasone (corticosteroids to control pain and inflammation), gabapentin and pregabalin (anticonvulsants used to inhibit neurotransmission of pain signals) and complementary treatments utilizing acupuncture. Because medical therapy does not address the underlying abnormality, long-term success is rare.

Foramen magnum decompression (FMD) is the preferred mode of therapy for Chiari type I malformation in people. Surgery is often successful, but 8-30% of patients require re-operation, due to excessive scar tissue formation at the FMD site. A FMD procedure for CLM in dogs has been described; the success rate of this procedure is approximately 81%. Similar to humans, about 25% of canine patients require re-operation due to excessive post-operative scar tissue formation. It has been reported that worsening clinical signs associated with scar tissue impingement typically occur within 3 months of surgery. A cranioplasty procedure developed for Chiari type I patients at the Chiari Institute, Great Neck, NY, has substantially reduced the frequency of post-operative scar tissue compression at the FMD site. A modification of that procedure for dogs with CLM with long-term follow-up was recently reported as part of a large-scale study. Currently, 21 dogs with MRI confirmed CLM treated with FMD with cranioplasty and greater than 6 months follow up (median 11.2 months) have been evaluated. Seventeen of 21 dogs were Cavalier King Charles spaniels; the 4 remaining dogs were a Pug, a Chihuahua, a Pomeranian and a Maltese. Following the FMD procedure, a cranioplasty was performed as previously described. A skull plate is fashioned using titanium mesh and polymethylmethacrylate (PMMA), and fixed to the back of the skull, using the titanium screw heads as anchor posts for the PMMA. In a recent study, there were no intraoperative and only minor postoperative complications. All but one of the dogs experienced clinical improvement following the procedure, although one has required a second decompression distal to the cranioplasty secondary to scar tissue formation. Mean follow-up time was 1 year (range, 6 to 16 mos). In that study, 9 dogs had follow up MR scans after surgery to evaluate the effect of surgery on both syrinx length and volume. At a median 5 months (range, 2-13 months) the syrinx length and volume were decreased 20% and 26.7% respectively. Cranioplasty appears to be well tolerated in dogs with CLM and may contribute to the reduction in syrinx size. It should be noted that intermittent exacerbation of clinical signs associated with excitement can be expected in some patients. Results of a large scale study involving cranioplasty with long-term follow up will be helpful in determining what impact cranioplasty will have in the treatment of this condition. Results of surgery can vary between surgeons for a variety of reasons, most likely due to the magnitude of the decompression. There are no guidelines on a desirable level of decompression and in human patients, excessive decompression can be detrimental due to cerebellar slouching and require revision surgery, however, inadequate

decompression may not restore normal CSF dynamics. Recognition of additional abnormalities and proper treatment planning for dogs with craniocervical junction abnormalities is essential to long term success. Proper imaging including complete brain and spine MR imaging and skull CT is needed to completely assess these patients.

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Table 1. Clinical signs associated with CLM-SM in dogs.

|       |                                  |
|-------|----------------------------------|
| 85.5% | Cervical / cranial hyperesthesia |
| 61.9% | Scratching                       |
| 47.6% | Diminished menace response       |
| 14.3% | Generalized seizures             |
| 14.3% | Facial paresis                   |



Figure 1a. Normal Canine skull and cerebellomedullary junction

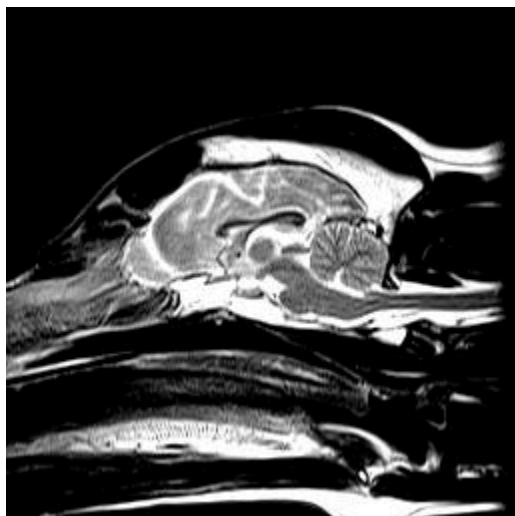


Figure 1b. Severe cerebellar compression (single arrow) with syrinx formation (double arrow) associated with Chiari like malformation.

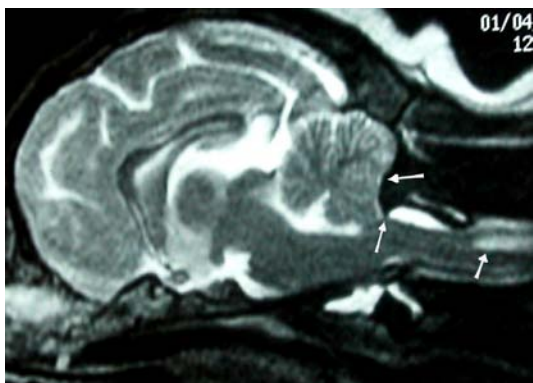


Figure 3  
Syringomyelia occurring on the thoracic and lumbar region.

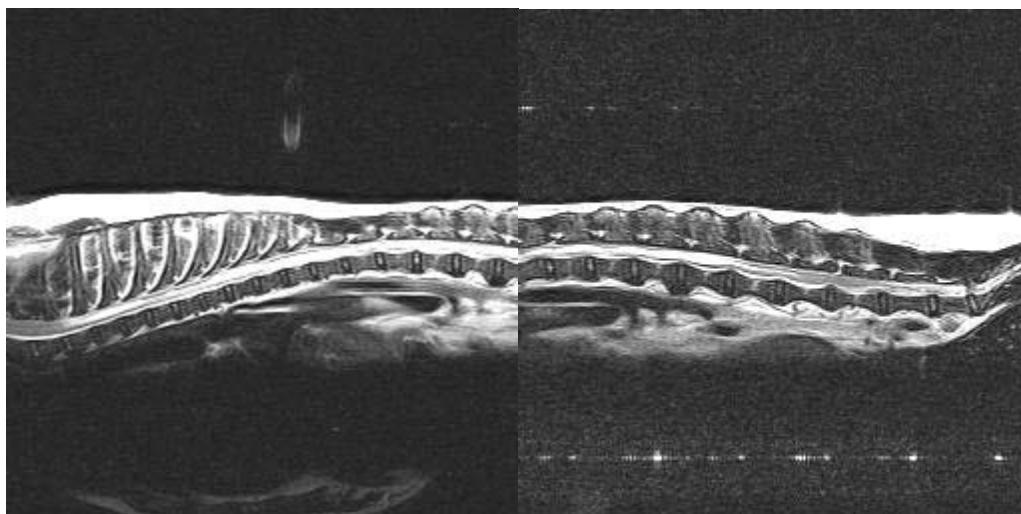


Figure 4  
Thermographic evaluation of dog with CLM

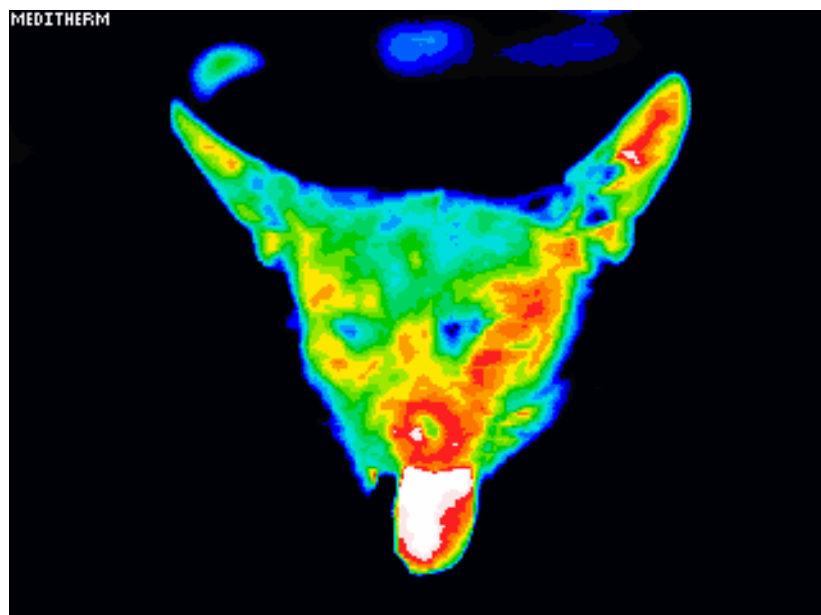


Figure 5  
Foramen magnum decompression with anchor screws in place in the occipital region.

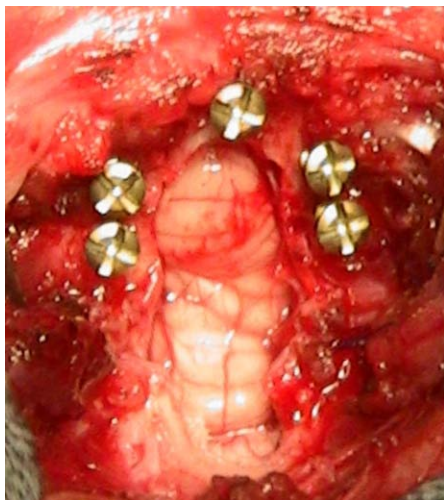


Figure 6a  
Cranioplasty formed from titanium mesh and polymethyl methacrylate



Figure 6b  
Spiral CT image of cranioplasty after surgery



## NOTES

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

# CLINICAL APPROACH TO THE KERATOSEBORRHOEIC PATIENT

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## INTRODUCTION AND DEFINITIONS

In the past 30 years, the study of seborrhoeic and scaling disorders has undergone three main stages. Firstly, P. Ihrke developed the notion of “seborrhoea”<sup>1-3</sup>. Secondly, Professor Escande at the GEDAC (French Dermatology Study Group) meeting in Paris on 13<sup>th</sup> March 1983 coined the term “keratoseborrhoeic state” (or syndrome) which was subsequently widely adopted by French-speaking dermatologists<sup>4</sup>. Thirdly, since work by K. Kwochka in the early 1990s<sup>5</sup>, the term “keratinisation disorder” has become fashionable. Fashions change but the condition remains the same: a chronic syndrome characterised by alterations in surface lipids (both sebaceous and epidermal) and excessive scale production. The relative importance of these varies from case to case but both are always present.

## ALTERATION OF SURFACE LIPID FILM

This skin film is composed of an emulsion derived from epidermal degradation products (mainly from the stratum corneum) and sebaceous gland secretion. The composition of sebum is complex and varies according to animal species (and possibly breed) and body region. In the dog, sebum is composed mainly of sterols (cholesterol), waxes (diesters) and free fatty acids<sup>1</sup>. Free fatty acids, present in very small quantities on normal skin, are derived from enzymatic hydrolysis of lipids by surface-living saprophytic bacteria. They are involved in regulation of skin pH which, in the dog, varies between 5.2 and 7.2. Skin pH is much more alkaline than in man, hence the use of topical agents of neutral pH in canine dermatology. Regulation of sebaceous gland secretion is complex and appears to be under hormonal control<sup>6,7</sup>. Dihydrotestosterone, a testosterone metabolite, is critical in activating mitosis and sebaceous secretions. The role of oestrogens, progesterone and corticosteroid hormones remains poorly understood. Besides hormones, hereditary factors are believed to play a part in this regulation. This may explain certain breed predilections for primary keratinisation disorders. Intrinsic and extrinsic (environmental) factors are also thought to be involved. The former are mostly metabolic and include linoleic acid, vitamin A (in the cat) and vitamin E. Environmental factors include temperature and humidity, which may influence sebaceous secretion ; dogs do not tolerate very high temperatures<sup>7</sup>.

The superficial lipid film has many roles<sup>6</sup>. In the normal dog, it ensures protection, cohesion and epidermal suppleness. It also plays the role of “lubricant”, imparting sheen to a normal coat. It renders the skin impermeable, particularly important in all the mucocutaneous regions where various watery secretions and excretions are emitted. It also provides chemical and thermal protection.

In keratoseborrhoeic disorders there is a reduction in waxes and a rise in free fatty acids and cholesterol<sup>1</sup>. The reduction in waxes and rise in free fatty acids lead to skin pH becoming more alkaline (8.2-8.6). To counteract this, more acidic topical agents are required. These changes in sebum promote the proliferation of skin bacteria which commonly cause secondary infections. The number of bacteria often increases 50 to 100-fold<sup>8</sup>. Their lipolytic activity further increases free fatty acid production leading to orthokeratotic (and sometimes parakeratotic) hyperkeratosis which, combined with accelerated epidermal turnover, contribute to the development of scale<sup>4</sup>. Scale, therefore, comes from both excessively produced keratinised cell layers and the sebum that binds them. Alteration of surface lipids is probably responsible for the smell of seborrhoeic dogs and cats. This rancid fat odour is quite characteristic, especially in greasy seborrhoea, and may be aggravated by secondary bacterial or fungal (e.g. *Malassezia pachydermatis*) infection. It can be obvious to the veterinary surgeon as soon as one of these dogs enters the consulting room. Indeed, the smell can be so embarrassing for a dog's owner that it becomes the principal reason for consultation.

Clinically, the following can be distinguished<sup>1-5,7,9,10</sup> :

- Dry seborrhoea (seborrhoea sicca): skin and hair are dry and waxy in appearance. After washing hands, there is a feeling of having candle wax on the tips of the fingers;

- Greasy seborrhoea (seborrhoea oleosa): skin and hair have a greasy appearance, sometimes even very greasy (oily); hairs may become matted in greasy tufts;
- Seborrhoeic dermatitis: lesions may be focal or multifocal. The characteristic lesion is a blackish, scaly, partially alopecic and lichenified macule, surrounded by a very scaly, annular zone of erythema, and/or bordered by an epidermal collarette. Bacterial infection and in some cases proliferation of *Malassezia pachydermatis* probably plays an important role in the formation of these lesions. Often seen in dogs with generalised greasy seborrhoea, these lesions frequently occur on the thorax, and may become generalised.

This classification is arbitrary and the three types may actually co-exist on the same animal. The same skin condition may also produce dry or greasy seborrhoea, depending on the case, possibly evolving from dry to greasy as the condition becomes chronic (e.g. flea allergy dermatitis, sarcoptic mange)<sup>3</sup>. On the other hand, some conditions tend to produce either dry (e.g. cheyletiellosis) or greasy seborrhoea (vitamin A-responsive dermatosis)<sup>3</sup>.

### SCALING (EXCESSIVE SCALE PRODUCTION)

Keratinisation involves a collection of morphological and biochemical processes which allow keratinocytes from the deep layers of the epidermis to transform (or differentiate) into cornified cells (corneocytes). It results in the formation of the cornified layer and bestows on the skin the role of epidermal barrier<sup>4,5,7,11</sup>. Good organisation of the cornified layer is ensured thanks to the lipid fraction, its corneocytes and its hydration level<sup>12</sup>.

Throughout this differentiation process, various biological and biochemical events are taking place<sup>5,13</sup>:

- Synthesis and expression of different keratins, assembled in macrofilaments by filaggrin, a protein matrix;
- Formation of a specialised, cornified cell envelope produced by polymerisation of specific proteins: involucrin, keratolinin, loricrin and filaggrin;
- Formation of an intercellular lipid mortar from neutral lipids and enzymes deposited by corneocytes, ensuring cohesion and corneal layer function.

This lipid mortar is the main barrier to transepidermal water loss. It is composed of ceramides and hydroxyacids forming lamellae between the cells (liquid crystals), and free fatty acids forming an intermediate layer<sup>13</sup>.

Keratinisation disorders are caused by alterations in proliferation, differentiation or desquamation or by a combination of some of these keratinisation abnormalities<sup>7,14</sup>. Keratinisation disorders can thus be split into two groups:

- Retention hyperkeratoses, associated with a corneocyte desquamation defect caused by biochemical changes to intercorneocyte cement lipids, following, for example, sebaceous gland destruction (leishmaniosis, granulomatous sebaceous adenitis);
- Proliferative hyperkeratoses, resulting from a surge of epidermal activity and subsequent defective keratinocyte maturation (differentiation), a feature of all inflammatory conditions (allergic skin conditions, ectoparasitic infestations, etc.).

Both can occur simultaneously. Some keratinisation disorders may however be caused by extensive epidermal necrosis, giving rise to large scale/crusts (e.g. erythema multiforme, auto-immune dermatoses, mycosis fungoides). Keratinocyte renewal takes on average 22 days in the normal dog<sup>11</sup>. In the cat, no studies on this have yet been published. In a canine keratinisation disorder this turnover rate is considerably reduced to 8 days<sup>11</sup>.

These disturbances in keratinisation have various clinical consequences for the seborrhoeic individual:

- Rapid, incomplete keratinisation leads to water loss. This makes the coat appear dull and is in part responsible for drying of the cornified layer; barrier function is no longer assured<sup>4,5,15</sup>;
- Enhanced release of membrane lipid into epidermal intercellular spaces increases disruption to the superficial lipid film<sup>16</sup>;
- Very rapidly shed corneocytes are abnormal in size and conformation, leading to grossly visible, variably sized, flakes of scale.

Clinically, excessive desquamation is the second component of keratoseborrhoeic dermatoses. Scale consists of white to greyish flakes depending on loss of cornified layer elements. Two types of scale have been described, according to size and thickness:

- Psoriasiform scale – large and relatively thick;
- Pityriasiform scale – small and thin.

Such a distinction, although clear in theory, may in practice be hard to make. Different sizes of scale may be present on the same animal. Furthermore, scale associated with the same skin condition (e.g. an allergic dermatosis) may initially be pityriasiform but later psoriasiform. In some conditions, however, scaling is definitely pityriasiform (e.g. demodicosis and colour dilution alopecia) and in others (e.g. cheyletiellosis and leishmaniosis), it is definitely psoriasiform.

Excessive keratin production also takes place within hair follicles. It may be associated with sebaceous hypersecretion and, in some cases, leads to **comedone and follicular cast** formation. Comedones are plugs of keratin and sebum which dilate the hair follicles. They are black if open, white if closed, and can be seen in many keratoseborrhoeic dermatoses including demodicosis, endocrinopathies and acne. Follicular casts, fragments of true scale which surround and adhere to the hair shaft, are less common. They are seen particularly in sebaceous adenitis (in which sebaceous glands disappear), ear margin seborrhoea, demodicosis and dermatophytosis<sup>3,17</sup>.

An **aetiological classification** is extremely useful in practice. It is the key to the clinical approach to the seborrhoeic patient. When the term seborrhoea was used, various, often very similar, classification systems distinguished primary from secondary seborrhoea. Secondary seborrhoeic disorders are linked to a particular, known condition which also manifests itself in other ways. Primary seborrhoeic disorders, on the other hand, are either caused directly by a metabolic disorder, or have no known underlying cause (idiopathic primary seborrhoea). Since the term keratinisation disorder has been in use, the distinction between secondary and primary disorders is approached in a different way in the dog. Secondary disorders are caused by an underlying dermatosis. In primary disorders, there is an apparent keratinisation defect for which the cause may or may not be known. Causes include alteration of the keratinocytes themselves, and retention hyperkeratosis. These conditions involve metabolic defects and are probably genetic in origin.

Secondary keratoseborrhoeic disorders include (dogs and cats):

- Ectoparasitic infestation (sarcoptic mange, cheyletiellosis, demodicosis, pediculosis and otodectes infestation)
- Allergic dermatitis (atopic dermatitis, food hypersensitivity, flea allergy dermatitis)
- Pyoderma
- Fungal infection (dermatophytosis, *Malassezia* dermatitis)
- Endocrinopathy
- Leishmaniosis
- Auto-immune and immune-mediated dermatoses
- Neoplasia (especially mycosis fungoides).

Primary keratoseborrhoeic disorders include (dogs):

- Primary idiopathic seborrhoea
- Follicular dysplasia (colour dilution alopecia, black hair follicular dysplasia and other dysplasias)
- Vitamin A-responsive dermatosis
- Zinc-responsive dermatosis
- “Epidermal dysplasia” or idiopathic hyperplastic dermatitis
- Sebaceous adenitis
- Lichenoid psoriasiform dermatosis and idiopathic lichenoid dermatitis
- Schnauzer comedone syndrome
- Ichthyosis
- Nasodigital hyperkeratosis
- Ear margin dermatosis



- Acne.

It must be emphasised that secondary disorders are far more common than primary disorders in dogs. In cats, primary keratoseborrhoeic disorders are even rarer than in dogs, and include exfoliative dermatitis, sebaceous adenitis, ichthyosis, and above all acne.

Age, breed and other clinical signs are important for the diagnosis of secondary keratoseborrhoeic disorders. Obviously appropriate complementary tests will be needed to establish the diagnosis. Age of onset of primary keratoseborrhoeic disorders is variable but as many of these diseases are genetic in origin, they appear in the puppy or young adult dog. There are definite breed predilections for primary keratinisation disorders<sup>3</sup>: Cocker Spaniels for primary idiopathic seborrhoea<sup>18</sup> and vitamin A-responsive dermatosis, West Highland White Terriers for idiopathic hyperplastic dermatitis, Nordic breeds for zinc-responsive dermatosis, Doberman Pinschers for colour dilution alopecia, Poodles and Samoyeds for sebaceous adenitis, and smooth-haired Dachshunds for ear margin dermatosis. After excluding secondary disorders by various appropriate tests, the confirmation of the primary disease shall be made by skin biopsies. In effect, dermatopathology will show compatible or more often diagnostic lesions<sup>19</sup>.

### TOPICAL THERAPY FOR THE KERATOSEBORRHOEIC PATIENT

Topical (locally acting) therapy is important in the management of many dermatological conditions, including keratoseborrhoeic disorders, because the skin is readily accessible to medications. Several formulations are available for the prescribing veterinary surgeon and may include many active ingredients. The active ingredients penetrate the skin through the intercellular spaces (lipophilic and hydrophilic molecules), through the epidermal cells (ion compounds) and above all through the hair follicles in animals, particularly for ionized molecules. Canine and feline skin is often more sensitive than is human skin due to anatomical and physiological differences, including differences in the thickness of the *stratum corneum* (thinner), skin pH (relatively more alkaline) and hair follicle density (higher) which can facilitate cutaneous penetration of active ingredients.

Traditional shampoo formulations are composed of **surfactants** (cleansing agents, foaming agents and conditioners) as well as thickeners, softeners, sequestering agents, preservatives, fragrance and sometimes opacifiers and colouring additives<sup>20,21</sup>. Surfactants are amphiphilic molecules, i.e. molecules with a dual affinity, both for water and oil. Surfactants are composed of a hydrophilic part (hydrophilic "head") and a lipophilic part ("lipophilic tail"). They are called surfactants (surface active ingredients) due to their propensity absorption on various interfaces (oil/water, air/water...), modifying the properties of the interface (decrease of the interface tension and stabilisation of the interface). Surfactants can be classified into 4 groups, according to their ionic nature: Anionic, Cationic, Non-ionic and Amphoteric which have various cleansing, foaming and conditioning properties as well as local tolerance. In water, surfactants form **micelle** structures. These structures correspond to a spontaneous molecular arrangement of the amphiphilic molecules: the objective is to minimize the contact of the surfactant lipophilic part with the aqueous environment. The micelle formation process explains many of the surfactant properties and particularly their ability to emulsify, render soluble and detach oils, dirt and debris, facilitating their elimination with the water. The emulsification process performed by surfactants is summarized in figure 1. The lipophilic part of the surfactant molecule surrounds the oily compounds (including debris) to form a micelle.

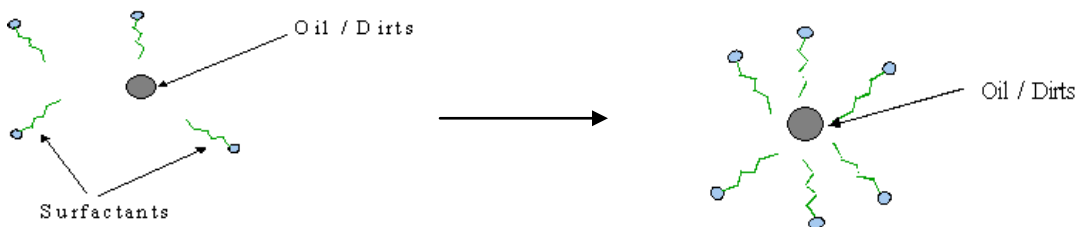


Fig. 1 Diagram of the emulsification process

Shampoos used to treat keratoseborrhoeic disorders contain particular agents and should be used appropriately<sup>22,23</sup>  
**Keratomodulating** agents work in two different ways<sup>7,20,22-24</sup> :

- Restoration of normal keratinocyte multiplication and keratinisation. Some of them exert probably a cytostatic effect on basal cells, thereby reducing their rate of division. Agents working in this way are called keratoplastic (keratoregulating) ;
- Elimination of excess corneal cells, by increasing desquamation (ballooning of corneocytes renders the *stratum corneum* softer and reduction of intercellular cohesion increases their shedding). Agents that work in this way are called keratolytic.
- 

**Salicylic acid** is a keratolytic agent. It causes a reduction in skin pH which leads to an increase in i) the amount of water that keratin is able to absorb. *Stratum corneum* hydration is therefore also increased ii) desquamation, via direct effect on intercellular cement and intercellular junction system (desmosomes). These actions help soften the corneal layer. Salicylic acid acts synergistically with sulphur, and is often present in small quantities in shampoos. Its efficacy varies with concentration.

**Coal tar** is a keratoplastic (cytostatic) agent. It reduces nuclear synthesis in the epidermal basal layers<sup>24,25</sup>. It is also antiseptic and antipruritic. There are many different sources and varieties of this active agent. Tar is a complex mixture of aromatic hydrocarbons, with many constituents (more than 10,000). It is hard to determine which is (are) responsible for therapeutic effects. Standardisation is therefore difficult, and good quality preparations must be used. Smell and consistency (with low foaming properties) of commercial preparations sometimes make it difficult to use, although deodorised veterinary preparations are available. Side-effects (e.g. skin drying, discolouration of pale coats, irritation and rebound effect) have been reported with high concentrations (over 3 %)<sup>20</sup>. Its use is contraindicated in the cat (neurotoxicity of benzopyrens, phenols and cresols). Tar is now prohibited in human cosmetics (because of toxicologic, teratogenic and photosensitization risks). Tar shampoos are replaced by other products (e.g. containing Melaleuca essential oil i.e. Tea Tree oil, zinc gluconate and vitamin B6). Tar shampoos are also abandoned in veterinary dermatology<sup>26</sup>.

**Sulphur** is mildly keratolytic. It forms hydrogen sulphide in the corneal layer and has numerous other, mainly antiseborrhoeic, properties (see below). It is also keratoplastic, due to a direct cytostatic effect and possibly because it interacts with epidermal cysteine to form cystine, an important component of the corneal layer<sup>7,10,24-25</sup>. It is also antiseptic but is drying. It exerts synergistic activity with salicylic acid. This synergism appears optimal when both substances are incorporated into the shampoo in equal concentration<sup>27</sup>. It is gradually being replaced in topical products by other more effective keratomodulating agents with fewer side-effects e.g. a rebound increase in seborrhoea: on cessation of effective therapy the condition may not only relapse but actually worsen.

**Selenium disulphide** is keratolytic and keratoplastic by reducing epidermal turnover and impairing disulphide bridge formation in keratin. It is also antiseborrhoeic (see below) but also has irritant and drying effects<sup>7,10,24</sup>. It too can cause rebound increase in seborrhoea and sometimes skin irritation. It is contraindicated in the cat.

**Ammonium lactate** has keratoplastic and keratolytic activity. In the management of human seborrhoea, it has been shown to be effective in reducing excessive scale by virtue of its keratoplastic activity<sup>28</sup>. Its mechanisms of action in keratoseborrhoeic disorders have not yet been completely elucidated but it seems to stimulate the living epidermis, correcting defects in keratinocyte multiplication and maturation. This facilitates terminal keratinocyte differentiation, leading to more normal desquamation<sup>29</sup>. Its properties are useful in keratoseborrhoeic disorders where it has important moisturising properties<sup>29</sup>. Several clinical studies in man indicate that this substance is very well-tolerated, even when used over prolonged periods<sup>28-29</sup>.

**Phytosphingosine** is a proceramid (ceramids are components of the extra cellular sheets of lipids in the stratum corneum) and a natural component of the epidermis, with anti-inflammatory and antimicrobial effects, used in human acne<sup>30</sup>. It promotes human keratinocyte differentiation<sup>31</sup>. An unpublished study suggests a comparable effect of a phytosphingosine-containing shampoo and an ammonium lactate-containing shampoo (Bourdeau PJ, Bruet V, Proc 21st NAVDF, Palm Springs, 2006, 174).

**Antiseborrhoeic** agents inhibit or reduce sebum production by the sebaceous glands, and help clear the ducts. **Sulphur** (see above) is a classic antiseborrhoeic agent, and may trigger a rebound effect. **Selenium disulphide** (see above) is antiseborrhoeic and may also cause a rebound effect.

**Benzoyl peroxide**, in addition to being antibacterial, is antiseborrhoeic, by hydrolysing sebum and reducing sebaceous gland activity. One study showed that 3% benzoyl peroxide shampoos increase transepidermal water loss and decrease skin surface lipid concentration and corneocyte counts (Campbell KL et al, Proc 10<sup>th</sup> AAVD/ACVD meeting, Charleston, 1994, 85). Benzoyl peroxide exerts a follicular flushing action which is very useful when treating comedone disorders and/or follicular hyperkeratosis<sup>10,24,32,33</sup>. Side-effects (irritations, erythematous rash) have been reported especially in concentrations above 5%<sup>5</sup>. The skin may also become dry and moisturisers are therefore always indicated after using this product.

**Zinc gluconate** has antiseborrhoeic properties. Zinc, as a type 1 5 $\alpha$ -reductase inhibitor, downregulates sebum production, and is used in human dermatology to treat acne vulgaris, both topically and orally<sup>34</sup>. **Vitamin B6** (pyridoxine) plays also a role in sebum secretion and there is a synergistic effect of unknown mechanism with zinc<sup>35</sup>. A veterinary shampoo containing salicylic acid, zinc gluconate, vitamin B6, linoleic/gamma-linoleic acids, piroctone olamine (an antifungal and antibacterial agent) and Tea Tree oil was proved as efficient as a shampoo containing coal tar, salicylic acid and sulphur in a randomized double blind study in the treatment of greasy keratoseborrhoeic disorders<sup>18</sup>.

Various veterinary shampoos have incorporated **essential fatty acids** for their softening and moisturising properties. One study has demonstrated that in seborrhoeic dogs, abnormal transepidermal water loss could be corrected by applying linoleic acid<sup>16</sup>. Some shampoos contain **moisturisers** such as glycerine, lactic acid and fatty acid polyesters. Moisturisers can be stored in multilamellar structures for prolonged release (Spherulites®), or mono/oligolamellar bodies (liposomes) to ensure continuing hydration effects.

Certain guidelines are suggested to use shampoos in keratoseborrhoeic disorders:

- A Long-haired dogs with severe seborrhoeic disorders may be clipped. Clipping leads to more effective application and better distribution of the active ingredient;
- Shampoos should initially be applied several times a week. With time, frequency of application can gradually be reduced to give the longest interval over which treatment is still effective, usually about 2 weeks;
- Cases should be monitored frequently. The therapeutic agent often needs to be changed following the development of side effects, rebound effects or change in clinical presentation (e.g. transition from greasy seborrhoea to dry seborrhoea).

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The more severe the dermatitis is, the more active and potent the shampoo must be and the more frequent will be the applications. For mild and/or pityriasiform keratoseborrhoeic disorders, keratolytic agents should be selected whereas for severe and/or psoriasiform disorders, keratoregulating (keratoplastic) agents will also be used. In all cases but particularly in greasy seborrhoea, antiseborrhoeic agents may be useful.

Clinical improvement is the main criterion in evaluating the **efficacy of shampoos**<sup>21,36</sup>. Their use has increased greatly in North America over the past 25 years, but they have been slow to gain acceptance in Europe<sup>10</sup>. However, they are now widely used in Europe, despite the fact that they were considered as contra-indicated and even harmful in the 60's. This dogmatism was a mistake and has probably delayed considerably the use of medicated shampoos, which are now regarded by the veterinary dermatology community as indispensable.

The efficacy of shampoos on skin hydration, the surface lipid film and *stratum corneum*, which are of great importance in keratoseborrhoeic disorders, can be evaluated objectively using a variety of techniques. These include transepidermal water loss (TEWL) measurement, corneocyte counts, measurement of corneal layer thickness, stripping, chemical analysis of lipid film, water content measurement, surface biopsy and corneometry<sup>37-38</sup>. In one study<sup>37</sup> corneometry, but not TEWL measurement, was found to give reproducible results. In another study, results from TEWL measurement, corneometry and sebometry were not reproducible and these procedures were therefore deemed to be useless in evaluating effects of topical treatments in the dog<sup>38</sup>. Electron microscopy could perhaps be useful<sup>39</sup>.

In recent years, there has been considerable progress in improving topical formulations, especially in prolonging the action of active ingredients applied to the skin. Micro-encapsulation in liposomes prolongs the moisturizing effect. Microencapsulation in multilamellar microvesicles such as Spherulites® increases bioavailability of therapeutic agents and promotes immediate and residual moisturising properties. Active agents are released by membrane rupture. Spherulite® surfactants are amphiphilic, with two antagonistic moieties – one hydrophilic, the other hydrophobic. They unite to form lamellar phases and are arranged in concentric layers using a specific manufacturing process. They are multilamellar, each membrane acting as a diffusion barrier to reduce loss of active ingredients to the external environment. They can act as a vehicle for a great number of active agents, hydrophilic or hydrophobic (lipophilic), released continuously and progressively at the surface of hairs and skin. This surfactant formulation is very useful in dermatology because it allows hydrophilic, active ingredients access to an oily environment and conversely hydrophobic, active ingredients access to an aquatic medium. The type of surfactant varies. In some cases (cationic surfactants), their charge is positive and Spherulites® attach preferentially to hairs and skin, whilst in other cases (non-ionic surfactants), the charge is neutral, allowing Spherulites® to penetrate the deeper skin layers. A study has demonstrated that non-ionic Spherulites® can penetrate the epidermis, hair follicles, sebaceous glands and dermis (Barthe N et al, Proc 16<sup>th</sup> ESVD/ECVD Congress, Helsinki, 1999, 156). The presence of chitosanide in cationic Spherulites® reinforces their positive charge and, by creating a film sheath over the hair, promotes excellent moisturising properties and prolonged effect.

**Lotions** are liquids in which active agents are dissolved or suspended. **Rinses** are concentrated and must be diluted before use. They are used after shampoos. Pump-sprays containing lotions are commonly used in canine dermatology (after or in between shampoos). These sprays might decrease the frequency of shampoos in certain cases. An anti-seborrhoeic micro-emulsion spray formulation containing phytosphingosine could replace the corresponding shampoo in a shampoo spray-sequence (3 shampooings followed by 2 sprayings vs 5 shampooings on a 15 day period – Bourdeau PJ, Bruet V, Proc 21<sup>st</sup> NAVDF, Palm Springs, 2006, 174). Microemulsions enhance bio-availability of active ingredients, which readily diffuse, and they also have an effective cleansing effect.

In every skin disorders, and in particular with dry seborrhoea, there is scope for increasing the humidity of the animal's skin, after shampooing, with a **moisturiser**. It has been demonstrated that skin hydration is less in dogs with scaling than in normal dogs<sup>15</sup>. Moisturisers lubricate, rehydrate and soften the skin. In the French language, they are all, incorrectly, lumped together as emollients. Moisturisers actually consist of true emollients, emulsifiers/emollients, occlusive dressings and rehydrating agents (= humectants)<sup>24</sup>.

In Europe only rehydrating agents are found in veterinary products. Emulsifiers/emollients and occlusive dressings are neither used nor marketed in the veterinary field, the latter due to risk of maceration. Emollients and humectants hydrate and soften the skin. They restore an artificial superficial skin film. Diluted in water, they can be massaged into the skin or applied as a lotion. Undiluted, they may be sprayed on after a shampoo. They should not be rinsed off. **Emollients**, containing lanolin alcohols, liquid paraffin or vegetal, animal or above all mineral oils, decrease transepidermal water loss<sup>7</sup>. They were borrowed from human dermatology and are now rarely used. Used as an emulsion in tepid water, they do improve coat condition, but also have a greasing effect which is a definite disadvantage. Local application of essential fatty acids has also been advocated to soften and rehydrate

the skin and reduce transcutaneous water loss<sup>16</sup>. No major occlusive effect is involved, and the effects are probably brought about by the incorporation of essential fatty acids (especially linoleic acid) into stratum corneum ceramides. **Humectants** have rehydrating and softening properties because of their hygroscopic properties (they attract water in the stratum corneum from the deep epidermis, dermis and also the environment if the relative humidity is above 70%)<sup>7</sup>. They reduce odour and improve coat appearance without the greasing effect. The high molecular weight of their active ingredients and their hygroscopic nature make them effective surface-protecting therapeutic agents. Examples include lactic acid, glycerine, propylene glycol, urea, colloidal oatmeal, sodium lactate and chitosanide. Propylene glycol is a diol alcohol. It is a colourless hygroscopic viscous liquid. It is a solvent but also a superior humectant and can induce keratolysis<sup>7</sup>. It is effective in managing sebaceous adenitis as a 50 to 75 % rinse or spray applied daily<sup>7,24</sup>. It has been also reported as effective at the concentration of 67 % in hereditary nasal parakeratosis in Labrador retrievers<sup>40</sup> and in naso-plantar keratoderma in Dogues de Bordeaux<sup>41</sup>. Two **spot-ons** have been recently proposed to treat keratoseborrhoeic disorders. One contains EFAs, essential oils and vitamin E. The second one contains phytosphingosine. The author is not convinced that this formulation is as effective as lotions and above all shampoos. Their advantage is their ease to use. It is the author's clinical impression that a new spot-on containing a lipid complex (ceramides, fatty acids and cholesterol) designed for atopic dermatitis could be helpful in some keratoseborrhoeic disorders (e.g. sebaceous adenitis).

**Gels** or **creams** can also be used in localized keratoseborrhoeic disorders. Petrolatum (petroleum jelly) is moderately effective for the treatment of hereditary nasal parakeratosis in Labrador retrievers<sup>42</sup>. Synthetic retinoids (tretinoin, isotretinoin and the more recent adapalene and tarazotene) are sometimes applied in a gel or cream as a treatment for localised lesions<sup>15</sup>. Tretinoin exists in lotion but is more used as cream. Isotretinoin gives relatively good results with localised comedone lesions (acne, nasodigital hyperkeratosis and ear margin seborrhoea)<sup>43</sup>. Main side-effects are photosensitisation and irritant reaction when applied too frequently<sup>1</sup>.

## SYSTEMIC THERAPY FOR THE KERATOSEBORRHOEIC PATIENT

Two **systemic synthetic retinoids** are particularly useful in the dog. Isotretinoin (13-cis-retinoic acid) may be given at a dose of 1-2 mg/kg/day for 2-3 months. acitretin is used at the same dose. Retinoids are vitamin A derivatives and have many biological properties. They regulate proliferation and differentiation of keratinocytes and produce a thinning of the cornified layer (inhibition of epidermal transglutaminases, corneal envelope formation, cholesterol sulphate synthesis, and collagenases; modulation of keratin expression)<sup>43</sup>. They reduce the size and output of sebaceous glands as well as having anti-inflammatory, immunomodulatory and probably antineoplastic activity<sup>44</sup>. With few documented cases involving retinoid treatment, results remain disappointing and inconsistent. Isotretinoin seems to be more effective for seborrhoeic conditions linked to hair follicle and sebaceous gland abnormalities whilst acitretin is better for proliferative hyperkeratotic conditions. Considering the cost of these products and their many possible side-effects (mucocutaneous junctional erythema, blepharoconjunctivitis, pinnal pruritus, digestive problems, hepatopathy, pancreatitis and above all, teratogenicity, which makes them completely contraindicated in breeding bitches), their use for now appears rather limited (Schnauzer comedone syndrome, sebaceous adenitis, lamellar ichthyosis, primary seborrhoea)<sup>44</sup>. Regular monitoring of animals treated with retinoids is required and should include Schirmer tear tests and full haematology and blood biochemistry panels. Two to three months treatment is essential before their efficacy can be determined. Oral **essential fatty acids** (EFAs) are considered by some authors as worthwhile for the treatment of seborrhoeic conditions in dogs<sup>7,22</sup>.

## CONCLUSION

A thorough understanding of the histopathogenesis and classification of keratoseborrhoeic skin conditions is essential. A careful history, detailed clinical examination and judicious choice of diagnostic tests always lead to diagnosis. Dermatopathology will be significant for the diagnosis of primary keratoseborrhoeic disorders. With this approach, the most appropriate and effective specific and/or symptomatic treatment may be prescribed. Topical therapy is mainly symptomatic and/or complimentary, and thus often used along with systemic treatments, mainly specific. Treatment and prevention strategies in veterinary dermatology often include the use of medicated shampoos, particularly in keratoseborrhoeic disorders. An appropriate formulation, judiciously selected active ingredients and the appropriate frequency of application make it essential. The prescription varies

according to each case and must take into account the nature and extent of the lesions, the concurrent specific treatment, the animal's temperament and willingness of the owner to devote the necessary time, and the concentration and potential side effects of the active ingredients. The therapeutic plan should be defined on both short and long term basis to obtain the best results, to enhance the owners' compliance and to limit potential side effects (Koch HJ, Proc WCVD3 Edinburgh, 1996, 88-90). Even with the tremendous recent progresses in companion animal dermatology, there is still a certain amount of art as well as science in devising the optimum topical therapy<sup>10</sup>. Communication is important and should underline the great value of medicated shampoos for the treatment of keratoseborrhoeic diseases of an animal with a haired skin.

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## NOTES

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# DERMATOPHYTOSIS IN DOGS AND CATS WITH EMPHASIS ON MANAGEMENT IN SHELTERS AND BREEDING FACILITIES

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## INTRODUCTION

Ringworm is a dermatophytosis involving an infection of the surface and infundibular keratine, hair shaft or claw. It is caused by fungi of the genus *Microsporum* and *Trichophyton*. In cats, ringworm is almost exclusively caused by *Microsporum canis* which can be enzootic in catteries and shelters. There is in fact a real predisposition of the feline species to infection with this dermatophyte. In dogs, *M. canis* is most often implicated but other dermatophytes can be isolated such as *M. gypseum*, *T. mentagrophytes* (in fact a complex including *Arthroderma vanbreuseghemii* isolated from dogs, cats and rodents, and *A. benhamiae* isolated from guinea pigs<sup>1</sup>), and *M. persicolor* which does not invade the hair shaft. If the dermatophyte is contracted from wild rodents, the dermatophytosis is called sylvatic. This is the case with *A. vanbreuseghemii*, *M. persicolor* and rarer dermatophytes (e.g. *T. erinacei*). Although in fact relatively uncommon, ringworm is a cause for concern as it is a potential zoonosis.

## CLINICAL SIGNS AND DIAGNOSIS

The clinical signs of dermatophytosis are highly variable and are not restricted to the classical nummular ringworm. Other aspects include localized, regional or generalized areas of alopecia, keratoseborrhoea and crusting on various localizations on the body surface including the face and ear pinnae, extremities and tail. Rarer clinical presentations include papules, pustules, kerion, onyxia and peri-onychia, cellulitis (in dogs), miliary dermatitis (in cats), dermatophytic mycetoma and acantholytic pustulosis. As a general rule, pruritus is very low, except in case of feline miliary dermatitis.

Any suspicion of ringworm after compilation of the medical history and performance of a complete clinical examination should always be confirmed (or excluded) by means of a rigorous clinical and laboratory diagnosis. This should primarily be based on four additional examinations (examination under a Wood's lamp, direct examination, histopathology and, above all, a fungal culture)<sup>2</sup>. Indeed, treatment is often difficult and therapy should not be used for diagnostic purposes<sup>3</sup>.

## FUNGAL CULTURES

The culture media used to identify ringworm are Sabouraud medium and DTM, which was proposed by Taplin in 1969.<sup>4,5</sup> These media offer equivalent diagnostic qualities with regard to dermatophytes of both human<sup>4</sup> and animal<sup>5</sup> origin. However, it is obviously necessary to confirm the presence of *M. canis* by examining the culture under a microscope, even in the absence of a change in colour of the DTM medium (according to Carroll,<sup>5</sup> a change in colour is observed only 82% of the time with dermatophytes of animal origin). One study showed that the use of an incubation temperature between 24°C and 27°C for the DTM enabled faster colour changes and sporulation, which helps to reduce the number of false negatives produced when cultures are left at room temperature.<sup>6</sup>

In shelters and breeding facilities the sample-taking procedure should last at least thirty seconds per animal, using a toothbrush, a sterile carpet square or a dust-catching cloth. It should be performed over the entire body, finishing with the face and ears or any lesions present.

## RATIONALE FOR THE TREATMENT OF DERMATOPHYTOSIS

Clinically, ringworm can be spontaneously self-curing in cats in about 4 months<sup>7</sup> and in puppies in about 2 months.<sup>8</sup> This is associated with the development of an effective immune response.<sup>9</sup> However, treatment and prevention are necessary for obvious ethical reasons and, also, to prevent contagion to humans or animals. This is particularly true for cats living in groups i.e. in a cat sanctuary or in breeding facilities.

## TOPICAL TREATMENT

Topical treatment of dogs and cats affected by ringworm is based on the clipping of infected hair and the application of a topical antifungal treatment twice a week.<sup>2,3</sup> In the vast majority of cases, topical treatment must be applied to the entire body surface. It should not be used without concurrent systemic treatment in infected animals, as it is potentially ineffective when used alone and may even aggravate the infection by causing it to become chronic.<sup>7,10</sup> It helps reducing the infectious load and can shorten the time to clinical and/or mycological cure.<sup>3,11</sup> The most effective products are enilconazole (available in the EU and Canada), shampoos and lotions containing miconazole and chlorhexidine (shampoo available in the United Kingdom and the USA, lotion available in the USA) and lime sulphur (available in the USA).<sup>3</sup> Despite of anecdotal reports of fatal idiosyncratic reactions in cats following exposure to enilconazole<sup>12</sup>, it seems that this product is safe for cats.<sup>13</sup> It is licensed for use in this species in France.

## SYSTEMIC TREATMENT

The most effective systemic agents to treat ringworm are griseofulvin, ketoconazole, itraconazole and terbinafine but the latter is not licensed in veterinary medicine.<sup>3</sup> Griseofulvin is licensed for the treatment of dermatophytosis in dogs and cats in many countries. Microsize griseofulvin is preferable and should be given at the dose of 50 mg kg<sup>-1</sup> divided BID, in conjunction with a fat meal to be better absorbed. Side effects (digestive, hepatic, haematological, neurological and cutaneous) are well known and appear to be linked to an individual idiosyncrasy rather than a dose-related toxicity but it is possible that pure-bred cats, particularly Himalayan, Abyssian and Siamese are more susceptible. Ketoconazole is licensed for the treatment of dermatophytosis in dogs in several European countries and is well tolerated in this species at the dose of 10 mg kg<sup>-1</sup> SID given during an acid producing meal. The recommended dose of itraconazole, a highly lipophilic triazole now licensed for use in cats in the EU, is 5 mg kg<sup>-1</sup> SID administered every other week, as justified by pharmacokinetic studies.<sup>14</sup> Secondary effects (nausea, anorexia and hepatotoxicity) occur above this dosage and are spontaneously reversible.

Systemic treatment is contra-indicated (griseofulvin) or not recommended (other drugs) for pregnant animals (because of teratogenicity) or nursing bitches and queens. As topical treatment alone is insufficient, isolation is advisable until weaning. Animals are treated normally afterwards. Breeding should be stopped in case of dermatophytosis. Kittens and puppies should be treated as adults, at least as soon as 8 weeks old. Before 2 months of age, isolation is recommended, until treatment is possible. However very young kittens can legally be treated with itraconazole, provided a precise dosage is given.

## TREATMENT OF THE ENVIRONMENT

*M. canis* spores can survive up to 18 months in the environment at room temperature in the presence of light.<sup>15,16</sup> In a house with a cat affected by ringworm, there may be up to 1000 *M. canis* spores per cubic metre of air.<sup>17</sup> Ringworm-infected cats (particularly kittens) heavily contaminate their environments, with a significant presence of viable airborne fungal elements.<sup>18</sup> Consequently, the environment must be disinfected at the same time as the cats, as it is a major source of exposure and recontamination, probably equal in this regard to asymptomatic carriers. Careful mechanical cleaning (vacuuming and use of detergents) reduces the load and optimises disinfection, as hair and organic waste bind with the spores.<sup>19,20</sup> Most of the *in vitro* studies on the effectiveness of the disinfectants concluded that an approximately 5% solution of pure household bleach, that is, sodium hypochlorite, a 1% formalin solution and enilconazole were effective,<sup>12,19-21</sup> whereas other options (among them, chlorhexidine and Virkon<sup>®</sup> S) were not. The most effective products are lime sulphur (primarily used topically in North America), diluted bleach (up to 1:20) and enilconazole (2% or 20 mL L<sup>-1</sup>), the latter continuing to be more effective even when diluted well beyond the manufacturer's recommendation.<sup>20,22</sup> A special enilconazole solution formulation (Clinafarm<sup>®</sup>, Janssen Cilag Animal Health), with surfactant properties that increase the contact between the spores and the product, is used in poultry farms to help prevent aspergillosis and has recently been approved in France to destroy dermatophyte arthrospores in the environment (homes with domestic pets). A specific French standard (Norme Française = NF in French) has been especially created for this product. The *in vitro* destruction rate is close to 100%, and the product does not stain. None of the above-mentioned products, except for pure bleach, has a prolonged residual effect (i.e., absence of growth in culture from samples taken 24 hours after application). Applications should thus be frequent, that is, somewhere between daily and two times a

week, based on an empirical calculation of maximum effectiveness, feasibility and cost.<sup>12</sup> It would appear that rinsing is not recommended. Enilconazole is also available in mist-making (fogger) format (Clinafarm<sup>®</sup>, Janssen Cilag Animal Health) and has a confirmed action (over 90% of hairs and cultures inactivated).<sup>23,24</sup>

### **DURATION OF TREATMENT**

Therapy should obviously not be stopped until all lesions are healed, and must continue at least one month after sampling for a culture which remains negative (ideally from all the animals in case of groups, particularly catteries). In studies, cultures are inoculated every 1 or 2 weeks and therapy is stopped after 2 or 3 negative cultures.

### **THERAPEUTIC PLANS FOR CATS LIVING IN GROUPS**

Immunosuppressed cats should probably be eliminated from such communities. Also, as part of the battle against the potential dissemination of spores, cats should be treated against ectoparasites (such as fleas, ticks, mosquitoes, *Cheyletiella* or *Otodectes* mites).

Theoretical recommendations have been proposed for the treatment and prevention of dermatophytosis in cats living in groups.<sup>2,15,25-29</sup> Cats with no lesions should be transferred to a separate area and cultures should be taken to detect contamination.<sup>11,25,26,30</sup> Animals with a positive culture should then be separated from animals with a negative culture, even if the former must thus be regrouped with the animals with lesions (i.e., a 2-area system, with one "clean" area). Ideally, the animals should be separated into 3 different areas: clinically-affected animals i.e. with ringworm lesions (by definition, with positive cultures), animals without lesions but with positive cultures ("asymptomatic infected subjects" or "mechanical carriers") and animals with negative cultures (i.e., a 3-area system, with one "clean" area).<sup>2,11,27</sup> The only exception to this model is that of nursing mothers, who must obviously remain in contact with their litter at all times and thus, must be isolated.<sup>26</sup> All movement between areas must be done in such a way as to prevent the spread of the infection (such as clean clothing, change of shoes, gloves).

Cats with a positive culture (clinically affected animals or subclinical carriers) must be treated topically and systemically, while healthy cats (negative cultures) must be isolated and can be treated topically as a precaution if considered as being preferable. However, if it is not possible to separate the animals, the most aggressive approach is to treat all animals topically and systemically, regardless of the specific fungal status of individual animals.<sup>2,25,28</sup>

### **PROPHYLAXIS**

Each newly introduced animal in a cattery should be sampled for culture (moquette, toothbrush or cloth technique) and quarantined at least one month, while culture must remain negative. Cats attending a show should be treated topically as soon as brought back home. Wood's light examination at the entrance of a cat show is useless since only 50% of the *M. canis* strains are positive.

### **PUBLISHED CLINICAL STUDIES**

Despite all the foregoing theoretical recommendations, which have most likely been implemented by their authors, few clinical studies on treating ringworm in groups of cats have been published<sup>10,30-36</sup> (Table 1). In 3 last studies, the shelters were completely free from infection in 49<sup>32</sup> to 56<sup>30,35</sup> days. In the most recent one<sup>36</sup> 73 % of cats were cured within 56 days, the remaining ones needing rescue therapy of up to 66 days.

### **CONCLUSION**

Diagnosis and therapy of canine and feline ringworm must be rigorous, being based on culturing, topical and systemic therapy, and disinfection of environment. The eradication and prevention of ringworm are possible in an animal rescue shelter, but at the price of demanding hygiene and expensive medication. This is also probably the case in breeding facilities.

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Table 1: Clinical studies on the treatment of dermatophytosis in groups of cats

| Reference                             | Number of cats/rooms | Systemic treatment        | Topical treatment (rinse)                    | Treatment of the environment | Outcome |
|---------------------------------------|----------------------|---------------------------|----------------------------------------------|------------------------------|---------|
| Guillot et al, 2002 <sup>31</sup>     | 100/1                | Lufenuron or griseofulvin | Enilconazole                                 | Enilconazole                 | Failure |
| Hnilica & Medleau, 2002 <sup>10</sup> | 22/1                 | None                      | Enilconazole                                 | Enilconazole (foggers)       | Failure |
| Fontaine & Bissot, 2005 <sup>32</sup> | 11/1                 | Itraconazole              | None                                         | Enilconazole                 | Success |
| Newbury et al, 2005 <sup>33-35</sup>  | 90 amongst >3600 /2  | Itraconazole              | Lime sulphur                                 | Gross cleaning Bleach        | Success |
| Carlotti et al, 2009 <sup>30</sup>    | 140/3                | Itraconazole              | Enilconazole                                 | Bleach and Enilconazole      | Success |
| Newbury et al, 2010 <sup>36</sup>     | 90/1                 | Itraconazole              | Lime sulphur (2) or miconazole-chlorhexidine | Bleach                       | Success |

## NOTES

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# FELINE FLEA AND TICK BORNE AGENTS

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The purpose of this manuscript is to provide a brief review of the emerging clinical issues associated with *Bartonella* spp, *Ehrlichia* spp., haemoplasma, *Anaplasma* spp. and *Rickettsia* spp. infections of cats.

Please also see the AAFP Panel report on feline bartonellosis [www.catvets.com](http://www.catvets.com), the ACVIM Consensus Statement on blood donor testing ([www.acvim.org](http://www.acvim.org)), and the ACVIM *Ehrlichia* Consensus Statement ([www.acvim.org](http://www.acvim.org)).

**Feline bartonellosis.** Cats have proven by culture or DNA amplification to be infected by *Bartonella henselae*, *B. clarridgeiae*, *B. koehlerae*, *B. quintana* and *B. bovis*. Cats are the main reservoir hosts for *B. henselae* and *B. clarridgeiae* and are likely to be the reservoir for *B. koehlerae*.

*Bartonella henselae* is the most common cause of Cat Scratch Disease as well as bacillary angiomatosis, and peliosis hepatis, common disorders in humans with AIDS. *Bartonella* spp. are thought to have both intra-endothelial and intra-erythrocytic phases of infection. Based on results of seroprevalence studies, culture, or polymerase chain reaction (PCR) assay, cats are commonly exposed to or infected by *Bartonella* spp.. Recently, *B. henselae* has been documented as a cause of chronic disease syndromes like fever, headaches and chronic fatigue in immunocompetent veterinary health care providers (Breitschwerdt et al, 2008). Most medical doctors may not recognize this differential and should be informed if you are exhibiting these problems.

The organisms are transmitted between cats by *Ctenocephalides felis* and so prevalence is greatest in cats from regions where fleas are common. In a recent study in the United States, we collected fleas from cats and attempted to amplify *Bartonella* spp. DNA from flea digests as well as the blood of the cat. The prevalence rates for *B. henselae* in cats and their fleas were 34.8% and 22.8%, respectively. The prevalence rates for *B. clarridgeiae* in cats and their fleas were 20.7% and 19.6%, respectively. Results are similar in other studies performed around the world including recent studies completed in England and Australia. In Scotland, we showed the *Bartonella* spp. seroprevalence and DNA prevalence rates to be 15.3% and 5.8%, respectively (Bennett et al, 2010).

*Bartonella henselae* survives in flea feces for days after being passed by infected *C. felis*. Infected flea feces are likely to contaminate cat claws during grooming and then *Bartonella* are inoculated into the human when scratched. It is also possible that open wounds are contaminated with infected flea feces. Thus, administration of flea control products, avoiding bites and scratches, and thorough cleansing of wounds or areas contaminated with flea feces is indicated to potentially decrease risk of acquiring bartonellosis.

We recently completed a study in which SPF cats were exposed naturally to *C. felis* (100 fleas added to each cat in R2 monthly) allowed to feed on cats with *B. henselae* administered IV. The barriers between R1, R2, and R3 were mesh so the fleas could move amongst the cats but the cats could not touch each other. The oblong circles represent the cat perches that were placed next to the mesh to encourage movement of fleas from group to group.

| R1                                                                                  | R2                                                                                   | R3                                                                                   |
|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| <ul style="list-style-type: none"><li>• 6 SPF cats</li><li>• Untreated</li></ul>    | <ul style="list-style-type: none"><li>• 6 cats with Bh</li><li>• Untreated</li></ul> | <ul style="list-style-type: none"><li>• 6 SPF cats</li><li>• Rx monthly</li></ul>    |
|  |   |   |
|                                                                                     |                                                                                      |  |



Cats in R3 were administered imidacloprid-moxidectin monthly and the other cats were not treated. At the end of the study, all cats in R1 had become infected but none of the cats in R3. We believe this data supports the Colorado State University recommendation that all cats used as blood donors be housed indoors when possible and be maintained on flea control products year round. At this time only imidacloprid-moxidectin has been shown to block transmission of *Bartonella* spp. amongst cats.

Most cats with serological evidence of exposure to a *Bartonella* spp., a *Bartonella* spp. cultured from blood, or microbial DNA amplified from blood by PCR assay are clinically normal. However, *Bartonella* spp. infection of cats has also been associated directly or indirectly with a variety of clinical manifestations like fever, lethargy, lymphadenopathy, uveitis, gingivitis, and neurological diseases. How often cats become ill from *Bartonella* spp. infections is unknown and more information is needed. For example, the association of *B. henselae* infection to uveitis in a cat was first made in an individual case with uveitis that ultimately responded to doxycycline therapy. We subsequently found *Bartonella* antibody production and DNA in the aqueous humor of cats previously presumed to have idiopathic uveitis. A series of clinical cases of feline ocular disease that were responsive to antibiotic therapy was recently reported. Thus, it appears likely that *Bartonella* spp. causes ocular disease in some cats. However, it can be difficult to determine which cats have been exposed and which cats are diseased. For example, in recent studies in my laboratory, the prevalence rates for *Bartonella* spp. antibodies in feline sera were not significantly different for cats with and without ocular disease, cats with or without seizures, or cats with or without stomatitis. It is also still unclear as to why some cats develop *Bartonella* associated illness and others do not. For example, we failed to induce *Toxoplasma gondii* or *Bartonella* spp. uveitis when we inoculated *Bartonella* IV into cats with chronic toxoplasmosis.

Blood culture, PCR assay on blood, and serologic testing can be used to assess individual cats for *Bartonella* infection. Cats that are culture-negative or PCR-negative and antibody-negative and cats that are culture-negative or PCR-negative and antibody-positive are probably not a source of flea, cat, or human infection. However, bacteremia can be intermittent and false-negative culture or PCR results can occur, limiting the predictive value of a single battery of tests. With PCR, false positive results can occur and positive results do not necessarily indicate that the organism is alive. While serologic testing can be used to determine whether an individual cat has been exposed, both seropositive and seronegative cats can be bacteremic, limiting the diagnostic utility of serologic testing. Thus, testing healthy, client-owned cats for *Bartonella* spp. infection is not currently recommended in the United States. Testing should be reserved for cats with suspected clinical bartonellosis. In our laboratory (<http://www.dlab.colostate.edu/>) we offer a combination of *Bartonella* spp. serology and PCR which I believe gives the best combined positive predictive values. If the results of *Bartonella* tests are negative in a clinically ill cat, the organism is not likely the cause of the clinical syndrome unless the infection was peracute and serological testing was used as the diagnostic test. If the results of *Bartonella* tests are positive, the agent remains on the differential list, but other causes of the clinical syndrome must also be excluded.

For blood donor cats, the ACVIM Panel was equivocal on their recommendation concerning blood donor testing of cats. In a recent study in our laboratory, we showed that *Bartonella* is not killed by CPDA-1 solution (Bradbury et al, 2010). Because of these findings and the fact that some cats become ill after IV inoculation with the organism, Colorado State University recommends only using *Bartonella* spp. PCR or culture negative and *Bartonella* spp. seronegative cats as blood donors.

In experimental studies, administration of doxycycline, tetracycline, erythromycin, amoxicillin-clavulanate, or enrofloxacin can limit bacteremia but does not cure infection in all cats. To date, use of antibiotics in healthy cats has not been shown to lessen the risk of cat scratch disease. Thus in the United States, treatment is generally recommended for clinically ill cats. If clinical bartonellosis is suspected, the AAFP Panel Report recommends doxycycline at 10 mg/kg, PO, daily for 7 days as the initial therapeutic trial. In the United States, I have my doxycycline prescriptions formulated into a flavored suspension to avoid esophageal strictures. Using the drug twice daily as labeled in Australia is also acceptable and may increase the chance of eliminating bacteremia. 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 I still believe bartonellosis

is a valid differential diagnosis, I consider azithromycin or a fluoroquinolones as second choices. In my experience, *Bartonella* spp. positive cats that have failed to respond after administration of 2 different drugs with presumed anti-*Bartonella* activity generally have another cause of the clinical syndrome. There is no clinical utility in rechecking *Bartonella* serological test results in cats.

To lessen the likelihood of acquiring a *Bartonella* spp. infection from a cat, the following are adaptations of what is recommended to HIV-infected people and other cat owners by the Centers for Disease Control and the American Association of Feline Practitioners.

- Flea control should be initiated and maintained year-round.
- If a family member is immunocompromised and a new cat is to be acquired, adopt a healthy cat > 1 year of age and free of fleas.
- Immunocompromised individuals should avoid contact with cats of unknown health status.
- Declawing of cats is generally not required but claws should be trimmed regularly.
- Bites and scratches should be avoided (including rough play with cats).
- Cat-associated wounds should be washed promptly and thoroughly with soap and water and medical advice sought.
- While *Bartonella* spp. have not been shown to be transmitted by saliva, cats should not be allowed to lick open wounds.
- Keep cats indoors to minimize hunting and exposure to fleas and other possible vectors.

**Feline granulocytotropic anaplasmosis.** Cats have shown to be susceptible to *A. phagocytophilum* infection after experimental inoculation. In naturally exposed cats, DNA of *A. phagocytophilum* has been amplified from several countries including Sweden, Denmark, Ireland, and the United States (Bjoersdorff and colleagues, 1999; Shaw and colleagues, 2001; Lappin and colleagues, 2004). Morulae consistent with *A. phagocytophilum* have been detected cytologically in neutrophils of naturally infected cats in other countries including Brazil, Kenya, and Italy. Cats living in endemic areas are commonly seropositive. As in dogs, *A. phagocytophilum* is transmitted by *Ixodes* ticks and so infections of cats are likely to be most common in these areas. While rodents are commonly infected with *A. phagocytophilum*, it is currently unknown whether ingestion or direct contact with rodents plays a role in *A. phagocytophilum* infection of cats. While the pathogenesis of disease associated with *A. phagocytophilum* in cats is unknown, some cats experimentally inoculated with *A. phagocytophilum* developed anti-nuclear antibodies and increased IFN-gamma mRNA suggesting that an immune pathogenesis of disease may contribute to the clinical findings.

Fever, anorexia, and lethargy were the most common clinical abnormalities. Tachypnea has also been detected. Ticks may or may not currently be infesting infected cats. Overall, clinical signs associated with *A. phagocytophilum* infection in cats were mild and resolved quickly after initiating tetracycline therapy.

Approximately 50% of cats with proven clinical infections induced by *A. phagocytophilum* have a mild thrombocytopenia (66,000-118,000/ $\mu$ l). Neutrophilia with a left shift, lymphocytosis, lymphopenia, and hyperglobulinemia have been detected in some cats. Morulae are less commonly detected than in dogs. The abnormalities resolved quickly after doxycycline treatment was initiated (Bjoersdorff and colleagues, 1999; Lappin and colleagues, 2004). Biochemical and urinalysis abnormalities are unusual. Some commercial laboratories offer serologic testing. Infected cats are negative for antibodies against *E. canis* and so *A. phagocytophilum* IFA slides should be used. Approximately 30% of cats with proven clinical infections induced by *A. phagocytophilum* are seronegative when first assessed serologically, but all proven cases to date have ultimately seroconverted. Some mountain lions with *A. phagocytophilum* DNA amplified from blood have been serum antibody negative (Foley and colleagues, 1999) and so a single negative antibody result in an acutely infected cat does not exclude infection. Therefore, cats with suspected anaplasmosis may need convalescent serum samples to prove infection. Alternately, antibody testing could be combined with PCR testing of whole blood in acute cases (Lappin and colleagues, 2004).

Supportive care should be administered as needed. Several antibiotics have been administered to naturally infected cats, but all cats in 2 studies became clinically normal within 24 to 48 hours after initiation of tetracycline or doxycycline administration and recurrence was not reported (Bjoersdorff and colleagues, 1999; Lappin and colleagues, 2004). While clinically normal, 2 cats were still PCR positive 17 days and 90 days after treatment (of 21 to 30 days duration) which suggests that treatment with tetracyclines for 21 to 30 days may be inadequate for eliminating the organism from the body (Lappin and colleagues, 2004)..

To prevent *A. phagocytophilum* infection in cats, acaricidal products that are approved for use on cat should be used. It is likely that *A. phagocytophilum* can be transmitted by blood; therefore, cats used as blood donors in endemic areas should be screened for infection by use of serum antibody tests or PCR assay and positive cats should be excluded as donors.

**Feline monocytotropic ehrlichiosis.** *Ehrlichia*-like bodies or morulae have been detected in peripheral lymphocytes or monocytes of naturally exposed cats in a number of countries including the United States, Kenya, France, Brazil, and Thailand. Two studies of naturally infected cats have amplified DNA consistent with *E. canis* (Breitschwerdt et al, 2002, Beaufils et al, 2002). Other studies of cats in endemic areas (Florida and Arizona) have failed to amplify *Ehrlichia* spp. DNA from the blood of cats (Luria and colleagues, 2004; Eberhardt and colleagues, 2006). To our knowledge, there have only been two experimental inoculation studies of cats with monocytotropic *Ehrlichia* spp. (Dawson and colleagues, 1988; Lappin and Breitschwerdt, unpublished observations, 2007). Morulae of *N. risticii* were detected in mononuclear cells from two of six cats inoculated IV (but not SQ); diarrhea developed in one cat, and depression, anorexia, and lymphadenomegaly developed in the other. When cats were inoculated SQ with an *E. canis* strain (North Carolina State University canine isolate) maintained in cell culture, microbial DNA or antibodies that reacted to *E. canis* morulae were not detected in an 8 week follow-up period. (Lappin and Breitschwerdt, unpublished observations, 2007). These results indicate the *E. canis*-like DNA amplified from naturally-infected cats may be from a different *Ehrlichia* spp. more infective to cats, not all *E. canis* strains will infect cats, not all cats are susceptible to infection by *E. canis*, or SQ inoculation is not an effective method for infecting cats with *E. canis*.

Sera from cats have been assessed for *Ehrlichia* spp. antibodies using IFA or western immunoblot. However, standardization of methodologies between laboratories has not been performed, the most appropriate cutoff values have not been determined, and there is variable serological cross-reactivity among *Ehrlichia* spp. *Neorickettsia* spp. and *Anaplasma* spp.. Therefore, results of serological studies should be interpreted cautiously. By use of IFA, serum antibodies that react with *E. canis* morulae have been detected in cats from multiple states in the United States, France, Italy, and Kenya. While antibodies have been commonly detected in naturally exposed cats, DNA of *Ehrlichia* spp. are rarely amplified from blood. When taken together, these results suggest that cats are less susceptible to monocytotropic ehrlichial infections than dogs.

It is currently unknown how cats are exposed to monocytotropic ehrlichial agents. Documentation of arthropod exposure in proven cases has been variable. Pathogenesis of disease associated with monocytotropic ehrlichiosis in cats is unknown but is likely to be similar to that for *E. canis* infection of dogs.

All ages of cats have been infected; most cats were domestic short haired, and both males and females have been affected. Anorexia, fever, inappetence, lethargy, weight loss, hyperesthesia or joint pain, pale mucous membranes, splenomegaly, dyspnea, and lymphadenomegaly were the most common historical and physical examination abnormalities. Dyspnea, petechiae, retinal detachments, vitreous hemorrhages, and pale mucous membranes were other reported physical examination abnormalities. Concurrent diseases are rarely reported but have included hemoplasmas (previously *Haemobartonella felis*), *Cryptococcus neoformans*, feline leukemia virus and feline immunodeficiency virus infections, and lymphoma.

Anemia is common and is usually nonregenerative. Leukopenia; leukocytosis characterized by neutrophilia, lymphocytosis, monocytosis; and intermittent thrombocytopenia were reported in some cats. Bone marrow evaluation of cats with cytopenias has revealed primarily hypoplasia of the effected cell line. However, one cat had bone marrow cytologic characteristics consistent with myeloid leukemia (Breitschwerdt and colleagues, 2002).

Hyperglobulinemia was reported in multiple cats; protein electrophoresis usually reveals a polyclonal gammopathy. An epidemiologic link has been made between the presence of *Ehrlichia* spp. antibodies in serum and monoclonal gammopathy (Stubbs and colleagues, 2000). Based on the cases reported to date, ehrlichiosis should be considered on the differential list for cats with unexplained leukocytosis, cytopenias, and hyperglobulinemia. Biochemical abnormalities were infrequently reported in cats with suspected monocytotropic ehrlichiosis and were non-specific. The three cats with *E. canis*-like DNA in blood also had antinuclear antibodies, similar to results reported for infected dogs (Breitschwerdt and colleagues, 2002).

Some cats with suspected clinical ehrlichiosis seroreacted to *E. canis* or *N. risticii* morulae. Antibodies that seroreact to more than one ehrlichia are sometimes detected. Some cats with *E. canis*-like DNA in blood were seronegative (Breitschwerdt and colleagues, 2002). In contrast, most *A. phagocytophilum* infected cats have strongly positive antibody test results. Positive serologic test results occur in both healthy and clinically ill cats, and so a diagnosis of clinical ehrlichiosis should not be based on serologic test results alone. A tentative diagnosis of clinical feline ehrlichiosis can be based on the combination of positive serologic test results, clinical signs of disease consistent with *Ehrlichia* infection, exclusion of other causes of the disease syndrome, and response to anti-rickettsial drugs. *Ehrlichia* spp. has been cultured from some cats on monocyte cell cultures. PCR and gene sequencing can also be used to confirm infection and should be considered the tests of choice at this time. However, as for dogs no standardization exists among laboratories providing *Ehrlichia* spp. PCR assays.

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

While cats and people can both being infected *E. canis*, direct transmission is not known to occur. Care should be taken when removing ticks, and arthropod control should be maintained at all time for cats, particularly if allowed outdoors.

**Feline hemoplasmosis.** The new names for *Haemobartonella felis* are *Mycoplasma haemofelis* (Mhf), ‘*Candidatus Mycoplasma haemominutum*’ (Mhm), and ‘*Candidatus M. turicensis*’. Strains evaluated in the United States, Australia, and the United Kingdom are genetically similar. In at least two studies of experimentally infected cats, Mhf is apparently more pathogenic than Mhm; all Mhf inoculated cats became clinical ill whereas Mhm inoculated cats were generally subclinically infected. Cats with chronic Mhm infection had more severe anemia and longer duration of anemia when experimentally infected with Mhf when compared to cats infected with Mhf alone.

In a recent study, we collected fleas from cats and attempted to amplify hemoplasma DNA from flea digests as well as the blood of the cat. The prevalence rates for Mhf in cats and their fleas were 7.6% and 2.2%, respectively. The prevalence rates for Mhm in cats and their fleas were 20.7% and 23.9%, respectively. Results from our collaborative study in Australia were similar (Barrs et al, 2008).

*Ctenocephalides felis* ingest Mhm and Mhf from infected cats when feeding. In one cat, we documented flea feeding to transfer Mhf. However, when we fed Mhf or Mhm infected fleas to cats, infection was not documented. In other studies, hemoplasmas have been transmitted experimentally by IV, IP, and oral inoculation of blood. Clinically ill queens can infect kittens; whether transmission occurs *in utero*, during parturition, or from nursing has not been determined. Transmission by biting has been hypothesized. DNA of the hemoplasmas has

been amplified from the mouths of cats, the salivary glands, and the tonsils. We are currently studying the role mosquitoes may play in the transmission of these agents.

Red blood cell destruction is due primarily to immune-mediated events; direct injury to red blood cells induced by the organism is minimal. Clinical signs of disease depend on the degree of anemia, the stage of infection, and the immune status of infected cats. Coinfection with FeLV can potentiate disease associated with Mhm. Clinical signs and physical examination abnormalities associated with anemia are most common and include pale mucous membranes, depression, inappetence, weakness, and occasionally, icterus and splenomegaly. Fever occurs in some acutely infected cats and may be intermittent in chronically infected cats. Evidence of coexisting disease may be present. Weight loss is common in chronically infected cats. Cats in the chronic phase can be subclinically infected only to have recurrence of clinical disease following periods of stress.

The anemia associated with hemoplasmosis is generally macrocytic, normochromic. Chronic non-regenerative anemia is unusual in cats with hemoplasmosis. Neutrophilia and monocytosis have been reported in some hemoplasma-infected cats. Diagnosis is based on demonstration of the organism on the surface of erythrocytes on examination of a thin blood film or PCR assay. Organism numbers fluctuate and so blood film examination can be falsely negative up to 50% of the time. The organism may be difficult to find cytologically, particularly in the chronic phase. Thus, PCR assays are the tests of choice due to sensitivity. Primers are available that amplify a segment of the 16S rRNA gene common to both hemoplasmas. Real time PCR to quantify hemoplasma DNA has now been titrated and can be used to monitor response to treatment. Since hemoplasmosis and primary immune hemolytic anemia are difficult to differentiate, cats with severe, regenerative hemolytic anemia are often treated with glucocorticoids and antibiotics.

Doxycycline has fewer side-effects than other tetracyclines in cats and so is preferred. I usually administer doxycycline as a flavored suspension (to avoid esophageal strictures) at 10 mg/kg, PO, every 24 hours for 7 days. If there is a positive response and the cat is tolerating the drug, I continue treatment for 28 days. If autoagglutination is evident, I generally prescribe prednisolone at 1 mg/kg, PO, every 12 hours for the first 7 days or until autoagglutination is no longer evident. Tetracyclines utilized to date appear to lessen parasitemia and clinical signs of disease but probably do not always clear the organism from the body and so recurrence is possible. In cats intolerant of doxycycline, enrofloxacin given at 5 mg/kg, PO, every 24 hours for 14 days was tolerated by cats and is equally effective or more effective than doxycycline. Administration of marbofloxacin or orbifloxacin gives similar results. Azithromycin was not effective for the treatment of hemoplasmosis in one study. Imidocarb administered at 5 mg/kg, IM, every 2 weeks for at least 2 injections was used successfully in the management of five naturally-infected cats that had failed treatment with other drugs. Blood transfusion should be given if clinically indicated. Most drug protocols have failed to eliminate infection and so at this time there is no clinical utility to repeat PCR testing. The owners should be warned that recurrences may occur.

To attempt to prevent hemoplasma infections, it might be prudent to control fleas. Cats should be housed indoors to avoid other potential vectors and fighting. Blood donor cats should be screened by PCR assay prior to use.

**Feline rickettsiosis.** *Rickettsia spp.* are obligate intracellular gram negative bacteria that are divided into two distinct groups, the spotted fever group (SFG) and the typhus group. Cats can be infected by *Rickettsia felis* and have been shown to have antibodies against *R. rickettsii*. *Rickettsia felis* was originally detected in a commercial cat flea (*Ctenocephalides felis*) colony and has been shown to belong in the SFG. Fever, headache, myalgia, and macular rash in humans have been attributed to *R. felis* infection in several countries around the world. In addition, one person in Mexico developed neurological symptoms following *R. felis* infection, suggesting that the organism may be the cause of severe debilitating disease in some people. The organism has been detected in *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 *C. felis* collected from cats in the United Kingdom, France, Israel, New Zealand, Australia, Thailand, and the United States.

In recent study in our laboratory, we assayed 92 pairs of cat blood and flea extracts from Alabama, Maryland and Texas, using PCR assays that amplify a region of the citrate synthase gene (gltA) and the outer membrane protein B gene (ompB). Of the 92 pairs, 62 of 92 (67.4%) flea extracts and none of the cat blood samples were positive for *R. felis* DNA. We have now documented *R. felis* DNA in fleas from cats in Australia (Barrs et al, 2008). In another study, we showed *R. felis* and *R. rickettsii* antibody prevalence rates in cats in the USA with fever to be 5.6% and 6.6%, respectively but neither organism was amplified from blood. These results prove that cats are sometimes exposed 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. Prevention in cats and people should include flea control. Since cats are generally not PCR positive for these organisms in blood, it is not currently recommended by Colorado State University to test blood donor cats.

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## NOTES

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# DERMATOLOGY IN CATTLE, SHEEP, GOATS AND PIGS

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Within the last 20 years, articles about skin conditions of cattle, sheep, goats or pigs have not overwhelmingly populated the animal health literature. The incidence of those conditions, nationally or globally, is difficult to track and treating them may occupy a small portion of a practitioner's clinical activity. Health of the skin of those species is no less important to the animal or the owner than it is with other species. It is the author's speculation that the relative difference in attention reflects the availability of products approved for the treatment of such conditions. Just as with other species, dermatologic conditions of cattle, sheep, goats and pigs, can be classified into etiologic categories: parasitic, bacterial, fungal, viral, nutritional, traumatic, neoplastic, immunologic or genetic.<sup>1-4</sup> Diagnostic procedures are similar regardless of species, while differential and etiologic diagnoses may be different in some instances. Few products are approved, specifically for the treatment of dermatologic conditions in animals intended for use as food. Management practices are usually recruited to reduce or eliminate the predisposing factors if not the direct causes of dermatologic conditions of those species. This presentation is not intended to be an unabridged discussion of those diseases; instead, it serves as a reminder of the role of healthy skin in those species and of potential career opportunities.

Most ectoparasitic infestations are species-specific and effective medications are available to treat many of those conditions. Some bacterial infections may be zoonotic. *Staphylococcus aureus* is ubiquitous and can pass from people to animals, or *vice versa*, and can be found in abscesses or other lesions of the skin. *Erysipelothrix rhusiopathiae* that causes erysipelas in pigs may also infect people as the cause of "erysipeloid." *Corynebacterium* or *Arcanobacterium* species are frequently found in skin lesions of those animals. Infections by *Dermatophilus congolensis* (dermatophilosis) are not uncommon during extended rainy weather, but there is no specifically approved treatment. While the names "interdigital dermatitis" or "interdigital necrobacillosis" ("footrot"), clearly indicate involvement of the skin, those bacterial conditions are of greater clinical concern when they progress to include deeper anatomic structures. Antimicrobial treatments and management adjustments are available to manage those conditions. In endemic areas, *Bacillus anthracis* may be an incidental "contaminant" and those organisms on the skin or hair of animals may be a source of incidental exposure of people who handle those animals or their hides. Dermatophytes may be primary pathogens or "secondary" to nutritional or immunologic deficiencies. While effective treatments are available, not all are approved for use in animals intended for use as food; correcting underlying predisposing conditions is important to improve chances of successful outcome and to prevent occurrence in the future. Some of those dermatophytes are zoonotic.

Viral diseases that express dermatologic lesions are frequently reportable diseases because of the similarity of lesions associated with "exotic" diseases (ex. Foot-and-Mouth Disease). Viral papillomas have not been eliminated. A biopsy of skin is the tissue of choice to diagnose a "non-dermatologic" viral disease of cattle; persistent infection with non-cytopathic Bovine Viral Diarrhea virus (BVDv).<sup>5</sup> When exposed to the non-cytopathic BVDv *in utero* before the fetus is immunocompetent, the fetus becomes immunotolerant to the virus which propagates in the fetus. Some of those persistently infected (PI) calves are born with no clinical signs of infection, show no ill effects and continue to shed the virus in skin cells or secretions of their body and serve to propagate the disease as a source for exposure of other naïve animals in contact.

Nutritional conditions of the skin can occur when attention does not consider specific management conditions of the producer. Traumatic skin lesions usually initiate a search for the cause and corrective measures are implemented. Neoplastic conditions of relatively common occurrence include squamous cell carcinoma and melanoma. Lesions of immunologic origin most often occur in association with exposure to immunogens in biologic or antimicrobial products. Animal skins of high quality from the USA contribute to the global leather and hide industry.<sup>6</sup>

Dermatology of animals used for food may not be considered as a clamorous career. Opportunities exist if one considers the importance of zoonotic pathogens, the importance of handling the skin/hide at the time of slaughter to prevent contamination of food, monitoring of pathogens and diseases that are important for national security (animal and human), animals for investigation of neoplastic or genetic diseases of people and the magnitude of the global market for leather/leather products. When treating those animals, one must be aware of and practice within the limits of the Animal Medicinal Drug Use Clarification Act (AMDUCA).<sup>7</sup>

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## NOTES

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.

# WHAT IS EVIDENCE-BASED MEDICINE?

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Doesn't everyone use "evidence" to make clinical decisions in their respective practice? How "strong" is that evidence? How can you know if it is reasonable to expect to improve chances of successful treatment; or of greater preventive protection? Risk assessment can be used to better understand results of research, to communicate those results with clients and colleagues, and to help develop realistic expectations resulting from clinical decisions while limiting bias or emotion. Evidence Based Medicine (EBM) is the approach to making clinical decisions on the basis of integration of two components: 1) clinical expertise of the attending veterinarian, and 2) the best clinical evidence from research.<sup>1-3</sup> Individual clinical expertise is the development of proficiency and judgment from experience. The best evidence from research focuses on clinically-relevant findings primarily, and preferably from patient-centered research. The emphasis of EBM has been on acquiring, assessing, and utilizing the *best research evidence*. A well-designed and executed randomized, controlled, blinded, clinical trial (RCT) is the highest standard of evidence for an individual study of treatment or preventive interventions. Results of well-designed, observational, prospective epidemiologic studies (i.e., patient- or other population-based cohort studies) also yield high-quality, valuable results.<sup>1,3</sup> Evidence Based Medicine has been transformed beyond a laudable *principle* to a *methodology* that consists of 5 steps: asking a clinically relevant question; searching for relevant evidence; critically appraising the evidence; applying the evidence to the patient(s); assessing the outcome. Critical appraisal comprises 3 steps: assessing the validity of the study (i.e., is the study methodology sound with respect to design, conduct, and analysis); assessing the clinical importance of the study; and, determining if results of the study are relevant to one's patient(s). Thus, if results of RCTs or cohort studies are internally valid it is essential to determine whether those results are *clinically important* and expressed quantitatively. Quantitative values for control event rate (CER) and principal event rate (PER) reported for RCT studies can be used to calculate quantitative values for relative risk (RR), relative risk reduction (RRR), absolute risk reduction (ARR) and number needed to treat (NNT). Those 4 quantitative values provide a measure of the magnitude of difference that can be expected and can be used to develop proper expectations. No one of those alone is "best" – certain measures will be best for certain circumstances or individuals. All of these measures can be applied to observational studies as well as randomized trials.

Veterinarians are trained, through years of education and experience, to understand and work within biologic systems that follow the laws of nature (ie. animals). Evidence Based Medicine and quantitative risk assessment can help develop realistic clinical expectations for quantitatively measureable responses to animal health decisions. The mathematical component is simple (1, 3, 4) and should not be minimized; however, the author considers most important, the critical evaluation of the research data and the proper interpretation of those data. Based on a report by O'Connor et al., we have areas in which we can improve. 5

## FORMULAE: (1, 3, 4)

Relative Risk (RR) = PER/CER or CER/PER;

Relative Risk Reduction (RRR) = |CER-PER| / CER = RRR; or, 1 – RR, where RR < 1;

Absolute Risk Reduction (ARR) = CER-PER;

Number Needed to Treat (NNT) = 1/ARR

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## NOTES

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# EVIDENCED-BASED TREATMENT OF CANINE DEMODICOSIS

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Demodex mites are transmitted from the dam to the pups in early life and normally do not pose a health problem. However in some dogs excessive proliferation of these mites leads to clinical disease. Predisposing factors for such proliferation includes genetic make-up, stress, endoparasites, malnutrition and debilitation in young dogs and underlying neoplastic or hormonal diseases in adult dogs. Such predisposing factors should be recognized and addressed as far as possible to optimize treatment efficacy. Published studies on the treatment of dogs with generalized demodicosis typically are rarely randomized and in general not placebo-controlled. One possible reason for this lack of a placebo group is that treating a dog with generalized demodicosis presented to a veterinarian with placebo is considered unethical by most veterinarians. Thus, at this point it is not known how many dogs with generalized demodicosis go in remission without treatment. As the endpoint of almost all studies is a microscopic remission of the disease, it is relatively easy to compare results of the various studies. However, different studies in different locations may be influenced by climatic differences as well as differences in the genetic make-up of the included patients. Almost all publications evaluating treatment of generalized demodicosis are in form of case reports or case series, but overall results achieved with the various drugs are very similar.<sup>1</sup>

## LOCALIZED DEMODICOSIS

This is a mild disease that usually heals spontaneously in 6 to 8 weeks but may wax and wane in a localized area for months.<sup>1,2</sup> Anecdotally, there is no difference between treated and untreated cases. If a therapeutic intervention is desired, mupirocin or benzoylperoxid gel can be gently massaged into the alopecic area once daily. Possible endoparasites should be treated and the general health status should be checked very strictly. In return visits, the veterinarian can determine whether there are any indications of generalized demodicosis. I do not treat the localized form at all with ectoparasiticides.

## GENERALIZED DEMODICOSIS

Although the prognosis of demodicosis has improved dramatically over the last decades, successful treatment may still be a challenge in some cases. About 90% of patients can be cured, but it may take up to twelve months of treatment.<sup>1</sup> However, the average time to clinical and microscopical remission in reported studies is approximately 2-4 months. The most common problem in the treatment is premature cessation of therapy.

Dogs with juvenile-onset disease may not always need miticidal therapy. However, there are no good studies evaluating, how many young dogs with generalized demodicosis recover spontaneously and anecdotal estimates vary widely from 0 to 50%. In intact females ovariohysterectomy is strongly recommended, because the dog may deteriorate or the disease may recur. In dogs older than 1 to 2 years of age or dogs with adult-onset generalized demodicosis systemic disorders can be the cause of demodicosis and the reason for an unfavorable treatment response.<sup>3</sup> Some dogs may recover without miticidal therapy if the underlying disease is treated successfully.<sup>4</sup>

Dogs with generalised demodicosis need to be reevaluated typically every 2 to 4 weeks and skin scrapings need to be obtained to monitor success of therapy. To determine the efficacy of treatment, the skin scrapings should always be done at the same sites.<sup>5</sup> If there is no clinical improvement or if skin scrapings show the same high number of mites or especially immature stages such as nymphs, larvae or eggs, change of treatment should be considered.

Secondary pyoderma is almost always present in dogs with demodicosis. The most common bacterial isolate is *Staphylococcus intermedius*. Empirical therapy for 3-8 weeks is usually appropriate. The most common gram-negative bacteria found in canine demodicosis are *Pseudomonas aeruginosa* or *Proteus mirabilis*. If rods predominate, bacterial culture and sensitivity is recommended, as the resistance pattern of these organisms is more unpredictable. In addition to systemic antibiotics, antibacterial shampoo therapy is frequently used to

remove crusts and treat bacteria topically. Benzoyl peroxide shampoo is most commonly recommended because of its presumed follicular flushing activity.

Commonly used miticidal therapies include amitraz and macrocyclic lactones such as ivermectin, moxidectin and milbemycin oxime.

### **AMITRAZ**

Amitraz is licensed for the treatment of generalized demodicosis. Some studies show clinical cure rates up to 90% with special amitraz protocols. Dogs with medium-length or long coats should be clipped to allow the solution to contact the skin and penetrate the hair follicle better.<sup>6</sup> All crusts should be removed. Before using amitraz the entire dog is washed with a medicated shampoo designed to kill bacteria and remove scales. Amitraz is then applied by wetting and sponging. The solution must be applied to the entire body. Adverse effects such as respiratory problems have been observed in humans, so the washing procedure should be performed outside or in a well-ventilated area and protective gloves should be worn.

The recommended treatment protocol in the USA is 0.025% amitraz used every 2 weeks. Higher concentrations and/or frequencies seem to be associated with a higher success rate.<sup>7,8</sup> Daily therapy with 0.125% amitraz on alternating halves of the body was reported to be efficacious for dogs with generalized demodicosis resistant to conventional therapy.<sup>9</sup> Amitraz at 1.25% weekly was used successfully to treat generalized demodicosis in eight dogs that had failed to respond to amitraz at lower concentrations.<sup>7</sup> These dogs were premedicated with atipamezole at 0.1 mg/kg intramuscularly once and yohimbine 0.1 mg/kg once daily orally for a further 3 days in an attempt to counteract the  $\alpha$ 2-adrenergic activity of amitraz which results in bradycardia, a decrease in rectal temperature and hyperglycemia. In patients with pododemodicosis or demodectic otitis externa a mixture of amitraz and paraffin or mineral oil has been recommended topically daily to every three days.<sup>1,2</sup>

Adult-onset demodicosis may respond less favourably to therapy.<sup>10</sup> Adverse effects with amitraz therapy were depression, sleepiness, ataxia, polyphagia/polydipsia and vomiting and diarrhoea.<sup>1</sup> With 1.25% amitraz, generalized erythema, scaling and an unpleasant odour were noted.<sup>7</sup>

Avermectins (e.g. ivermectin, doramectin) and milbemycins (milbemycin oxime and moxidectin) are antiparasitic agents produced by the fermentation of various actinomycetes. They bind selectively to glutamate-gated chloride channels resulting in increased cell permeability for chloride ions and cause neuromuscular blocking resulting in paralysis and death of the parasite. They also interact with gamma-aminobutyric acid (GABA) sites. Mammalian safety is due to mammals not having glutamate-gated chloride channels in the peripheral nervous system. In mammals, GABA is a central nervous system neurotransmitter and these drugs do not cross the blood-brain barrier.

### **MILBEMYCIN**

Milbemycin is licensed only for the use of monthly heartworm and intestinal parasite prevention in dogs at 0.5 mg/kg. A variety of dogs of all breeds, including the breed that are sensitive to high-doses ivermectin, and various ages at onset of diseases and onset of therapy were treated with dosages varying from 0.5 to 3.8 mg/kg given daily.<sup>11-14</sup> The mean duration to achieve negative skin scrapings was 8-26 weeks; mean treatment duration was 12-30 weeks.<sup>1</sup> Clinical cure rates varied from 15% to 92%, depending on the dosage used and the age at onset of disease.<sup>1</sup> Several studies have shown a better success rate with higher doses.<sup>11,13,15</sup> Dogs with adult-onset disease respond more poorly to treatment than dogs with juvenile-onset disease.<sup>11,13,15</sup> There are some rare adverse effects, including stupor, ataxia and trembling in two dogs at 3.8 mg/kg/day, transient vomiting and lethargy.<sup>1</sup>

### **IVERMECTIN**

Ivermectin is not licensed for treating demodicosis in any country to the author's knowledge. It is only licensed in small animals for the prevention of dirofilariasis at a dosage of 0.6 mg/kg once monthly. It was first reported as a treatment of generalized demodicosis almost 20 years ago. Dosages usually vary from 0.3 to 0.6 mg/kg/day.<sup>1</sup> An early protocol, treating with ivermectin at 0.4 mg/kg subcutaneously once weekly did not demonstrate much efficacy.<sup>16</sup> The mean duration time with daily therapy to achieve negative skin scrapings was 6.5 to 28 weeks, the

mean treatment duration 10-33 weeks. Higher dosing does not seem to influence the time to first negative skin scrapings.<sup>1</sup> The rate of clinical cure varied from 83% to 100% in the individual studies. The pour-on formulation of ivermectin, which is very effective in the treatment of nonfollicular mites of the dog and cat, performed very poorly when it was applied three times weekly at a dosage of 1,5 mg/kg to dogs with generalized demodicosis. Only 2 of 12 treated dogs achieved parasitological cure.

Side effects of ivermectin include lethargy, oedematous wheals, ataxia and mydriasis. These developed as late as 10 weeks into treatment. Collies are particularly sensitive. One report recommended to gradually increase the dose of ivermectin administered from 0.05mg/kg to 0.1, 0.2 and 0.3 mg/kg during the first days of treatment to identify sensitive animals.<sup>17</sup> Because of the relative long half-life of ivermectin, serum concentrations of ivermectin administered daily continue to increase for weeks before reaching equilibrium at much higher levels than with weekly therapy. Thus, chronic toxicity due to cumulative therapy may develop with prolonged daily ivermectin treatment.

Today, some veterinary laboratories offer gene test to recognise dogs with a defect of the MDR-1 gene encoding for the multi-drug-resistance-transport protein P-glycoprotein. This gene was reported to be associated with ivermectin toxicity in the Collie.<sup>18,19</sup> The MDR-1-defect is autosomal-recessive. However, in a recent study, 26 of 27 non-Collie dogs with chronic ivermectin toxicity had a normal MDR-1 gene!<sup>20</sup>

### **MOXIDECTIN**

Moxidectin is another avermectin and was used in three studies in a dose of 0.2 to 0.4 mg/kg/day.<sup>21-23</sup> Combining the studies, 52 dogs with generalized demodicosis, 41 with juvenile-onset and 11 with adult-onset demodicosis, were treated with oral moxidectin. Thirty one (76%) of the dogs with juvenile-onset demodicosis were in remission after 8-14 weeks, seven were lost to follow-up. Of the eleven dogs with adult-onset demodicosis, nine were in remission after 8-16 weeks. All dogs were cured, but details on long-term follow-up were unavailable. Transient side effects included lethargy, anorexia, ataxia, vomiting and stupor. More studies with longer follow-up period periods are needed to identify potential benefits and disadvantages of this drug. Moxidectin as part of a spot-on approved for the treatment of many endo- and ectoparasites including demodicosis has become recently available in many countries. Weekly application of the spot-on has a clearly better success rate than monthly application<sup>24</sup> and dogs with severe demodicosis seem to respond less favourably than dogs with mild disease.<sup>25</sup>

### **DORAMECTIN**

Doramectin is also a macrocyclic lactone. In one study, twenty-three dogs were injected weekly with 0.6 mg/kg subcutaneously for 5-23 weeks. Ten of the dogs were cured, seven relapsed after 1-24 months (two of which responded to repeat doramectin treatment) and six were lost to follow-up.<sup>26</sup> In another study, doramectin was given weekly or twice weekly orally at the same dose with good success.<sup>27</sup> More studies are needed to evaluate the efficacy and optimal dosing of doramectin for the treatment of canine demodicosis.

A number of other drugs were evaluated as treatment for canine demodicosis but at this point in time insufficient evidence for the treatment of canine generalised demodicosis is available for amitraz collars, closantel, deltamethrin, doramectin, herbal and homeopathic preparations, muramyl dipeptide, phoxime, vitamin E and fair evidence against the use of pour-on ivermectin, levamisole, lufenuron, oral selamectin and ronnel.<sup>1,28</sup>



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## NOTES

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# EVIDENCE-BASED THERAPY IN PEMPHIGUS FOLIACEUS

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## INTRODUCTION

Evidence-based medicine (EBM) or evidence-based practice (EBP) aims to apply the best available evidence gained from scientific method to clinical decision making. It assesses the strength of evidence of risks and benefits of treatments. Evidence-based medicine categorizes different types of clinical evidence and rates them according to the strength. The strongest evidence for therapeutic interventions is provided by systematic review of randomized, triple-blind, placebo-controlled trials with inclusion and follow-up utilizing a similar patient population and medical history. In contrast, patient testimonials, case reports, and even expert opinion have little value as proof because of the placebo effect, the biases inherent in observation and reporting of cases and difficulties in determining who is actually an expert. The most widely and accepted format to evaluate human and healthcare interventions is by the Cochrane Collaboration which is an independent organization that summarizes the effects of human healthcare interventions by means of regularly updated systematic reviews.[1] Many veterinary dermatology studies and clinicians are now applying and practicing evidence-based medicine. A good example of EBM was utilized by the International Task Force for Canine AD for therapies which should be recommended for canine AD. This publication graded high quality randomized controlled trials and systematic reviews based upon degrees of evidence and strengths of recommendation for a variety of different therapeutic approaches to treat dogs with AD. This paper modified an existing human grading scale to give tiered ratings based on the strength of the existing data in the literature.[2] Despite progress in utilization of evidence based medicine in veterinary dermatology, there is need for further evaluation, especially when we look at clinical trials regarding treatment interventions for pemphigus foliaceus.

Even in the human field where evidence-based medicine is well established when you examine the studies and data critically for the treatment of pemphigus foliaceus optimal information is lacking regarding the most effective and safest therapies. A recent review to assess interventions for efficacy and safety in the management of pemphigus vulgaris and pemphigus foliaceus was published.[3] In this Cochrane review the optimal glucocorticoid dose, the role of adjuvant immunosuppressive medications, and the long-term adverse events could not be clearly defined. It included reviewing the Cochrane Skin Group Specialized Register (October 2008), The Cochrane Central Register of Controlled Trials (The Cochrane Library Issue 4, 2008), MEDLINE (2003 to October 2008), EMBASE (2005 to October 2008), LILACS (1981 to October 2008), Ongoing Trials Registers, reference lists of articles, conference proceedings from international pemphigus meetings and experts in the field. From these resources randomized controlled trials of any intervention in pemphigus vulgaris or pemphigus foliaceus were collected and analyzed by two authors independently who assessed quality and extracted data. All investigators were contacted for further information. Adverse events were identified from included studies. The results of eleven studies with a total of 404 participants (337 pemphigus vulgaris, 27 pemphigus foliaceus and 40 not specified) were identified. The quality of included studies was not high, the majority of studies did not report allocation concealment, and power was limited by very small sample sizes. The interventions assessed included prednisolone dose regimen, pulsed dexamethasone, azathioprine, cyclophosphamide, cyclosporine, dapsone, mycophenolate, plasma exchange, topical epidermal growth factor and traditional Chinese medicine. Ten studies included participants with newly diagnosed or newly active recurrent disease, and one trial included participants in maintenance phase. There was sufficient data for 4 meta-analyses, each pooling results of two studies only. For the majority of interventions, results were inconclusive. The researchers found some interventions to be superior for certain outcomes, although were unable to conclude which treatments are superior overall. Mycophenolate was more effective in achieving disease control than azathioprine (1 study; n=40; RR 0.72; 95% CI 0.52 to 0.99, NNT 3.7). There was evidence of a steroid-sparing benefit of azathioprine (1 study; n=57; MWD -3919 mg prednisolone; 95% CI -6712 to -1126) and cyclophosphamide (1 study; n=54; MWD -3355 mg prednisolone; 95% CI -6144 to -566) compared to glucocorticoids alone. Topical epidermal growth factor decreased time to control (1 study; n=20; HR 2.35; 95% CI 1.62 to 3.41). The researchers concluded that there is inadequate information available at present to ascertain the optimal therapy for pemphigus vulgaris or pemphigus foliaceus. Further

research is required, especially to assess the optimal glucocorticoid dose, the role of adjuvant immunosuppressive medications, and long-term adverse events to improve harm: benefit analyses.

When reviewing the veterinary literature for treatment interventions in canine and feline pemphigus foliaceus, all recommendations were made based on interventions used in human disease and no controlled prospective clinical trials have been reported regarding treatments in animals. So when making the following recommendations for treatment interventions these are based on only two small open clinical trials and for the most part retrospective individual or case series studies. If we use a modification of a previously used category system that incorporated the Cochrane Skin Group for an overall grade of quality and scrutiny of the evidence of efficacy and harm based upon US Preventive Services Task Force (Table I), you can see we can only give a rating of category C: Open clinical trial or D: Cohort, case-control analytic studies, descriptive study, or case reports. And based on this the strongest evidence from the existing studies would be limited to insufficient evidence to recommend for/against the current therapies being utilized! However, this is what is currently available to evaluate our existing interventions for pemphigus foliaceus in dogs and cats.

**Table I\***

**Category of evidence of interventions**

**(A)** Blinded randomized controlled trial (control with either active drug or placebo);

**(B)** Controlled trial lacking either blinding or randomization

**(C)** Open, uncontrolled trial

**(D)** Cohort study, case-control analytic study, descriptive study, case report:

**(1)** > 50 subjects per group,

**(2)** 20–50 subjects per group,

**(3)** 10–19 subjects per group,

**(4)** < 10 subjects per group.

**Strength of recommendation of interventions**

1. More than one study, including at least one blinded RCT, supports the high efficacy of the drug tested, there is ‘good’ evidence ‘for’ recommending the use of this medication.
2. At least one blinded RCT provides support of medium to high efficacy of the drug investigated, there will be ‘fair’ evidence ‘for’ recommending the use of that drug.
3. Blinded RCTs are not available, or when multiple studies yield controversial evidence of treatment effect, it will be concluded that there is ‘insufficient’ evidence ‘for/against’ recommending prescription of the medication tested.
4. At least one blinded RCT provides evidence of lack of efficacy, or efficacy associated with common harmful events, there is ‘fair’ evidence ‘against’ recommending the use of this medication.
5. More than one study, including at least one blinded RCT, supports the lack of efficacy of the drug tested, or supports any efficacy but with unacceptable side effects, there is ‘good’ evidence ‘against’ recommending the use of the drug evaluated.

Modified from [4] Olivry, T. and R.S. Mueller, *Evidence-based veterinary dermatology: a systematic review of the pharmacotherapy of canine atopic dermatitis*. Vet Dermatol, 2003.

**Table II - List of canine and feline pemphigus foliaceus cases reports, prospective/retrospective case studies and open clinical trials**

| Study               | Type                  | Case # | Interventions                                                          | Outcome                                                                                                  |
|---------------------|-----------------------|--------|------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Halliwell (1977)[5] | Case Report<br>canine | 1      | Prednisolone                                                           | Partial response at daily dosing                                                                         |
| Manning (1982)[8]   | Case series<br>Feline | 3      | Prednisolone 2 cases<br>Dexamethasone and<br>Aurothioglucose in 1 case | 2 cases PF – maintained on<br>prednisolone<br>1 case PE- maintained on dex and<br>weekly aurothioglucose |

|                        |                         |    |                                                                             |                                                                                                                                                                                                                                                       |
|------------------------|-------------------------|----|-----------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Ihrke (1985)[6]        | Case Series<br>Canine   | 37 | Prednisone<br>Azathioprine,<br>Aurothioglucose                              | 39% responded to corticosteroid therapy alone, and 50% and 55% responded, respectively, to prednisone and cytotoxic drugs, and to prednisone with aurothioglucose. Aurothioglucose alone in 27% of the dogs. One-year survival was achieved in 53%    |
| Noxon (1989)[11]       | Case report<br>Canine   | 2  | Prednisone<br>Aurothioglucose                                               | 1 case controlled with glucocorticoids and gold salts                                                                                                                                                                                                 |
| White (1992)[16]       | Case report             | 8  | Tetracycline/niacinamide                                                    | 1/8 good 3/8 poor response                                                                                                                                                                                                                            |
| Scott (1997)[9]        | Case series<br>Feline   | 10 | Prednisone<br>Aurothioglucose                                               | 6/10 (60%) pred responsive<br>4/10 (40%) aurothioglucose responsive                                                                                                                                                                                   |
| Scott (1997)[9]        | Case series<br>Canine   | 26 | Prednisone<br>Cyclophosphamide<br>Azathioprine<br>Aurothioglucose           | 23 of 26 (88%) managed successfully single or combination of drugs                                                                                                                                                                                    |
| Byrne (2001)[14]       | Open prospective trial  | 8  | Mycophenolate mofetil                                                       | 3 cases with reduction of lesions and severity<br>4 cases did not complete and 2 dogs were euthanized<br>All required concurrent glucocorticoids to control lesions                                                                                   |
| Preziosi (2003)[10]    | Retrospective<br>Feline | 57 | Prednisone<br>Triamcinolone<br>Chlorambucil                                 | 44 cases followed for 1-54 m (median 9 m)<br>Triamcinolone more successful (15/15) inducing remission than prednisone (8/13) or prednisone with chlorambucil (9/11)<br>4/44 cats died from disease or therapy                                         |
| Olivry (2003)[13]      | Open prospective trial  | 5  | Cyclosporine                                                                | 1 case transient improvement<br>4 cases failed to complete trial due to lack of efficacy                                                                                                                                                              |
| Griffies (2004)[17]    | Case report             | 2  | Tacrolimus topical                                                          | 2 cases of facial pemphigus managed with no adverse reactions                                                                                                                                                                                         |
| Rosenkrantz (2004)[12] | Case review<br>Canine   | 31 | Prednisone<br>Triamcinolone<br>Azathioprine<br>Chlorambucil<br>Cyclosporine | 31 cases followed 1 to 5 y, mean of 2.7 years, survival rate of 22/31 (71%).<br>9 cases (29%) died, 5/9 unrelated to disease or treatment and 4/9 (44%) were euthanized due to poor response to therapy (2/4) or discontinuation of medications (2/4) |
| Olivry (2004)          | Case reports            | 6  | Prednisone<br>Azathioprine<br>Topical glucocorticoids                       | 6/51 (12%) cases reviewed went into long-term remission                                                                                                                                                                                               |

|                        |                     |    |                                                                  |                                                                                                                                                      |
|------------------------|---------------------|----|------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------|
| Gomez(2004)            | Retrospective study | 43 | Prednisone<br>Azathioprine                                       | 40% survival<br>Most dogs died in 1 <sup>st</sup> yr<br>69% (18/26) euthanized due to lack of response, poor quality or adverse effects of treatment |
| Rosenkrantz (2007)[15] | Case reports        | 3  | Glucocorticoids,<br>Azathioprine<br>Cyclosporine<br>Ketoconazole | Glucocorticoids were d/c within 3 – 12 weeks after the addition of cyclosporine<br>All cases were in remission (10 -18 mo)                           |
| Vaughan (2009)[18]     | Retrospective study | 40 | Antibiotics<br>Glucocorticoids<br>Azathioprine                   | 37/40 treated<br>27/37 (73%) remission<br>4/37 (11%) improved<br>3/37 (8%) no improvement<br>14/17 (82%) adverse reactions had tissue eosinophilia   |

## **CURRENTLY UTILIZED DRUG INTERVENTIONS[12]**

### **Glucocorticoids**

Localized forms of PF and PE can be treated with topical glucocorticoids. Occasionally topical therapy in conjunction with systemic therapy can be used in more persistent focal lesions. A potent glucocorticoid is often needed initially and if adequate response is seen switching to a less potent topical glucocorticoid is then recommended. Common options used by the author include 0.1% amcinonide cream (Cyclocort, Lederle) or mometasone (Momentamax, Schering Plough). Less potent formulations can also be effective and options would include 0.015% triamcinolone acetonide solution (GENESIS, Virbac) or hydrocortisone aceponate (HCA) (Cortavance, Virbac). These can be used daily for 7 days then EOD for 7 days and if an adequate response is seen an even less potent formulation can be tried (1 – 2 % hydrocortisone sprays, gels, creams, ointments (Corticalm, Cortispray DVM Pharmaceuticals, Resicort, Virbac and Generics) on an as needed basis.

The most common form of therapy used in pemphigus foliaceus management is systemic glucocorticoids. In the author's specialty referral practices, 35% of the PF cases are adequately controlled with only glucocorticoid therapy. The form of oral glucocorticoid therapy selected depends on the individual case response and the side effects seen in that particular patient. Most commonly prednisone or prednisolone is utilized at immunosuppressive dosages. Initial dosages at 2.2 – 4.4 mg/kg q 24h can be used. If response is seen within 10-14 days this dosage is gradually reduced on a daily basis over 30-40 days and then lowering to an alternate day basis with the ultimate goal of dosing at 1 mg/kg q 48h or less. The author prefers methylprednisolone (Medrol, Pfizer) to prednisone or prednisolone due to the reduced mineralcorticoid effects resulting in less polyuria and polydipsia. In addition there are some cases that will respond more favorably to methylprednisolone than prednisone or prednisolone. It is also the preferred from to use in cats, as oral prednisone is not very well absorbed and/or converted to prednisolone.[19] The dosing and tapering regime is the same as for prednisone or prednisolone. Oral triamcinolone (Vetalog, Fort Dodge) or oral dexamethasone (Azium, Schering-Plough and generics) are alternative glucocorticoids that can be utilized in more refractory cases or in cases that have profound polyuria and polydypsia or other behavioral or personality changes. Triamcinolone can be particularly effective in feline pemphigus. [10]These glucocorticoids are considered to be 6-10 times more potent than prednisone or prednisolone. Starting immunosuppressive dosages for these drugs range from 0.2-0.6mg/kg q 24h for triamcinolone and 0.2-0.4 mg/kg q 24h for dexamethasone. As with prednisone therapy these dosages need to be reduced gradually and eventually tapered to a q 48 h to q 72h basis. As these glucocorticoids suppress the hypothalamic-pituitary-adrenal axis for 24-48 hours, it is optimal to give these drugs every 72 hours for maintenance. However the author has had many cases do very well long-term on an every 48 hour basis as

maintenance. Maintenance dosages range from 0.1- 0.2 mg/kg q 48-72h for triamcinolone and 0.05 - 0.1mg/kg q 48-72h for dexamethasone.

In severe cases of pemphigus foliaceus, shock dosages of prednisolone sodium succinate (10mg/kg/IV) or dexamethasone (1mg/kg/ IV) can be utilized.[12, 20] This can be performed as a one time treatment or given two days consecutively. This can be followed with a modification of other oral glucocorticoid therapy. This form of therapy has a higher incidence of gastrointestinal ulceration, in particular gastric hemorrhage. Concurrent use of gastric protectants is usually recommended when this form of therapy is utilized.

Monitoring should include semiannual complete blood counts, chemistry profiles, urinalysis and urine cultures. If cases are non-responsive to glucocorticoids, fail to control on safe alternative day to every 72 hour dosing or have undesirable side effects, alternative or concurrent immunosuppressive drugs are indicated.

### **Azathioprine Therapy**

Azathioprine (Imuran, Glaxo Wellcome and generics) is the author's favorite and first choice immunosuppressive to add to glucocorticoids or use as an option to glucocorticoids to use in canine pemphigus cases. The author has not recognized any differences with brand name or generic therapy. It can be used as a glucocorticoid-sparing agent in cases when glucocorticoids cannot be reduced to safe long-term levels, used in combination with glucocorticoids or other immunosuppressives in more refractory cases, or as a sole therapy. Even though it is effective in the cat, it is generally contraindicated in cats due to its more profound myelosuppression and potential for fatal reactions in cats thought to be due to lower thiopurine methyltransferase levels.[21, 22] It is dosed at 1.5 – 2.5mg/kg or 50mg/m<sup>2</sup> q 24h - 48h in dogs. It is available as a 50mg scored tablet. It 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. It has a slow onset of action and usually takes 4-8 weeks to see clinical effects. Adverse reactions primarily include myelosuppression (lymphopenia, anemia and leukopenia) diarrhea and increased susceptibility to opportunistic infections when used long-term (pyoderma, demodicosis and dermatophytosis). The diarrhea that can be seen usually responds to dosage reduction, although it can be severe (hemorrhagic) and require drug discontinuation. Less common complications include vomiting, hepatotoxicity and possible pancreatitis. A rare azathioprine induced hepatotoxicity can be seen that usually responds to drug withdrawal. [23] 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 2-3 weeks for the first 3 months of therapy. Initially periodic (every 2-3 months) monitoring of chemistry profiles is also recommended. Once cases are in remission monitoring can be reduced to every 6 months.

### **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. It is dosed at .1 -.2-mg/kg or 15mg/m<sup>2</sup> q 24h to 48h. It is available in a 2 mg non-scored coated tablet, making dosing in small dogs and cats easier. Myelosuppression is a concern and similar monitoring as listed with azathioprine should be performed. Other side effects include vomiting, diarrhea and anorexia.[24] The author will use chlorambucil in the canine as a glucocorticoid sparing drug, as an alternative to azathioprine, in combination with glucocorticoids and azathioprine in more refractory cases or as a sole therapy in cases not tolerating other therapies. It is also a good option in feline pemphigus when glucocorticoids do not work or are not tolerated.[10, 25]

### **Tetracycline and Niacinamide**

The combination of tetracycline and niacinamide has been used with variable success in dogs and humans with pemphigus.[16] The author will commonly use this therapy but usually finds it an adjunctive therapy at best for the pemphigus complex. It may be more successful in localized cases such as pemphigus foliaceus limited to the face or pemphigus erythematosus. Tetracycline has anti-inflammatory properties affecting complement activation, antibody production, chemotaxis, prostaglandin synthesis, lipases and collagenases. Niacinamide inhibits mast-cell degranulation and phosphodiesterase. Adverse reactions include vomiting, diarrhea, anorexia and increased

liver enzymes. The author and others have seen rare cases of seizures in dogs on this combination of therapy, that responds to discontinuation of therapy. When gastrointestinal complications occur, discontinuing the niacinamide may reduce or eliminate these problems. In rare cases the tetracycline may still produce beneficial results. 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. Clinical response may take 1 –2 months. If clinical response is seen the frequency can be reduced to twice or even once daily.

### **Cyclosporine and Tacrolimus**

Cyclosporine (Neoral, Novartis) and tacrolimus (Prograf oral formulation and 0.03% and 0.1% Protopic topical preparation, Fujisawa USA, Inc) are immunosuppressant agents that have been evaluated for the treatment of autoimmune diseases. Both of these drugs work similarly, however tacrolimus is much more potent and the oral formulations appear toxic in the canine and 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. This results in blocking of regulatory proteins that upregulate activation genes of T-helper inducer and cytotoxic cells. Of the cytokines affected Interleukin-2 (IL-2) is most notably affected, although many other cytokines are affected. The initial studies of cyclosporine in the treatment of pemphigus and other cutaneous autoimmune diseases has not been impressive and only limited responses have been seen.[13, 26] However one of these studies utilized older non-microemulsion formulations of cyclosporine.[26] The author has seen individual case responses utilizing the microemulsion formulation (Atopica, Novartis). It is dosed at 7-10mg/kg q 24h often with ketoconazole 5mg/kg q 24h to aid increasing relative serum levels of the cyclosporine. It is also common to use cyclosporine in conjunction with oral glucocorticoids. However, it may be used as a sole agent where it has limited responses [13, 26] or as a glucocorticoid sparing agent. It has also been reported more recently to be used in conjunction with azathioprine in more refractory cases of pemphigus with good success. [15] The author and others have also used cyclosporine in cases of feline pemphigus foliaceus with success at 10mg/kg/d. Cyclosporine is also available as a topical 0.2% ointment (Optimmune, Schering-Plough) which can be utilized for localized forms of pemphigus. Generally more effective is the use of topical administration of 0.1% tacrolimus. Localized pemphigus may respond favorably to topical tacrolimus with no adverse reactions reported in one study. [17]

### **Mycophenolate Mofetil**

Mycophenolate mofetil (CellCept, Roche Pharmaceuticals) inhibits de novo purine (guanine) synthesis. B and T lymphocytes are dependent upon guanosine synthesis because they are unable to use the salvage guanosine synthesis pathway. This unique aspect of lymphocytes allows mycophenolate mofetil to inhibit the proliferation of lymphocytes and the production of antibodies relatively selectively with minimal effects on other tissues. In humans it has been used in a variety of autoimmune skin diseases. The main side effects include bone marrow suppression, nausea, vomiting, diarrhea, and increase incidence of infections. It does not have significant renal or hepatic toxicity. Canine studies show success rates of approximately 50% with some dogs weaned completely off prednisone while others have relapsed when the glucocorticoids were dropped too low. Dosages ranged from 22-39 mg/kg q 24h divided q 8h. Side effects were minimal but the most common included pyoderma and malassezia, diarrhea and leukocytosis. Expense is a limiting factor with brand name CellCept as a 50 pound dog will require therapy costing \$10/day.[14] However, newer generics are much less expensive and clinically appear to work as well as brand name.

### **Conclusion**

It is obvious from this review of evidence-based therapy for pemphigus foliaceus that prospective controlled studies are needed. Because of the need to treat patients with active drug therapy, placebo controlled studies would be difficult to perform. However comparing a prospective study with randomization to a glucocorticoid only group, compared to glucocorticoids combined with other immunosuppressives needs to be performed.



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## NOTES

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# **AN EVIDENCE BASED REVIEW OF THE USE OF LIMITED ANTIGEN DIETS AND OTHER TESTS AS AN AID TO THE DIAGNOSIS OF CANINE FOOD ALLERGY**

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## **Limited antigen diets**

A review of the literature regarding dogs with skin disease +/- other system involvement shows that publications regarding the use of limited antigen diets have employed these diets either in a) the identification of dogs with adverse food reactions (AFR, table 1) or b) to assess the tolerance of a test diet in dogs with confirmed AFR (table 2). For the purpose of this review publications involving client owned dogs have been analysed.

Assuming that the limited antigen diet trial (LADT) and rechallenge is the gold standard for the diagnosis of CAD, from a clinical standpoint the important questions are as follows;

1. How long should the diet be fed?
2. Which diet should be selected?
3. How quickly might clinical signs recur after challenge?

Regarding the length of time required to feed the diet a review of the literature, summarised in table 1, clearly shows a tendency over time for the period over which the diet is fed to increase. This might be attributed to clinical experience but is probably also influenced by a study performed by Rosser<sup>1</sup> (1993) in which he reported that 13/51 dogs required more than 6 weeks on a LADT to achieve maximal improvement. It should be noted that the majority of these studies include dogs with additional hypersensitivities such as environmental or flea allergies. Pruritus and clinical signs associated with these hypersensitivities might be expected to fluctuate daily or seasonally and thus could influence assessment of the end point of the diet trial itself.

Additional treatments are often given initially when a LADT is started and Chesney<sup>2</sup> (2002) qualifies the length of the diet trial itself to be dependent on this factor. Outcome measures are variable but in most cases a reduction in pruritus is required and sometimes quantified although the reports in which a percentage reduction is cited do not elaborate as to how this measurement was achieved.

The selection criteria for most studies is a response to a LADT thus dogs with variable cutaneous signs are often included and additional systemic signs (GI, Seizures, sneezing) may be present.

From this review a relapse of clinical signs within 14 days of challenge would be expected in the dog with AFR although it should be noted that in most cases the authors are relying on untrained client/ owner observation. For animals with IgE mediated food allergy one would expect the development of clinical signs within hours of challenge. Either the dogs included in these studies have heterogeneous disease or immediate signs are being missed. Many studies did not report a follow up period to determine whether an improvement was sustained after diagnosis and whether dietary compliance was maintained. This is important as this author's clinical experience suggests there are cases in which the initial diagnosis looked like AFR but the dog relapsed even on a strictly fed diet.

Client and pet compliance is a major consideration when performing a LADT. Palatability and cost were cited as negative points in a trial with a hydrolysed diet (Loeffler et al 2004)<sup>3</sup>. Whereas the compliance was significantly reduced in another study when owners were asked to home cook for their pet (Tapp et al. 2002)<sup>4</sup>

Regarding selection of a maintenance diet for those dogs diagnosed with AFR the studies cited in table 2 address this issue. Dogs included here have been diagnosed with AFR and are offered a limited antigen diet(s). In only one study (Beale and LaFlamme 2002) was it known whether dogs had previous sensitivities to the parent protein from which the new diet was derived. Whether previous exposure to proteins in the novel diets had been

the case was not always reported. Interestingly White (1986) reported 6 dogs which were fine on a home cooked lamb and rice diet but did not tolerate a commercial lamb and rice diet. In no study was the limited antigen diet tolerated by all dogs.

| Investigator            | Diet                                             | # dogs<br>(confirmed<br>diagnosis) | Duration<br>Of diet trial               | Outcome<br>measure                              | Relapse<br>time after<br>challenge | Follow<br>up             |
|-------------------------|--------------------------------------------------|------------------------------------|-----------------------------------------|-------------------------------------------------|------------------------------------|--------------------------|
| White<br>1986           | Home<br>cooked                                   | 29                                 | > 3weeks<br>or 2<br>weeks><br>treatment | 80-100%<br>decrease<br>pruritus or<br>other c/s | NR                                 | 6<br>months              |
| Carlotti et<br>al. 1990 | Home<br>cooked                                   | 33                                 | > 3 weeks                               | 70-80% ↓<br>pruritus                            | 12-72<br>hours                     |                          |
| Harvey                  | Home<br>cooked                                   | 25                                 | > 3 weeks                               | Clinical<br>improvement                         | 2-14 days                          |                          |
| Rosser<br>1993          | Home<br>cooked                                   | 51                                 | 1-10 weeks                              | Maximal<br>improvement                          | 1 hr-14<br>days                    | Several<br>months        |
| Denis &<br>Paradis 1994 | Home<br>cooked                                   | 73                                 | 1-13 weeks                              | ↓ pruritus                                      | NR                                 |                          |
| Patterson<br>1995       | Commercial                                       | 20                                 | 4-8 weeks                               | ↓ Pruritus<br>score                             | NR                                 |                          |
| Leistra et al.          | Home<br>cooked                                   | 40                                 | 6-10 weeks                              | 100% ↓<br>pruritus                              | 1-3 weeks                          | 6<br>months              |
| Chesney<br>2002         | Home<br>cooked                                   | 19                                 | 4-9 weeks<br>(treatment<br>dependent)   | Pruritus score                                  | < 10 days                          | 10 wks<br>– 12<br>months |
| Jeffers et<br>al.1991   | Home<br>cooked                                   | 13                                 | 3 weeks                                 |                                                 | < 5 days                           |                          |
| Tapp et al.<br>2002     | Home<br>cooked                                   | 8                                  | 6 -8 weeks                              | > 50%<br>improvement                            | NR                                 |                          |
| Jeffers et al.<br>1996  | Home<br>cooked                                   | 25                                 | 3-10 weeks                              | ↓ Pruritus                                      | <14 days                           | NR                       |
| Bourge et<br>al. 2003   | Hydrolyzed<br>commercial                         | 58 (36*)                           | 2 months                                | ↓Pruritus<br>score                              | NR                                 |                          |
| Loeffler et<br>al. 2004 | Hydrolyzed<br>commercial                         | 9                                  | 6 weeks                                 | ↓ Pruritus                                      | < 14 days                          |                          |
| Loeffler et<br>al. 2006 | Hydrolysed<br>commercial<br>Or<br>Home<br>cooked | 21                                 | > 4 weeks                               | ↓ Pruritus                                      | 2-7 days                           |                          |
|                         |                                                  | 35                                 |                                         |                                                 |                                    |                          |

Table 1. Diets employed in the diagnosis of canine AFR

\* Two dogs diagnosed with AFR but did not respond to the hydrolysed diet

| Investigator            | Diet                                         | # dogs                               | # dogs intolerant                      | Duration Of diet trial                        | Outcome measure          |                |
|-------------------------|----------------------------------------------|--------------------------------------|----------------------------------------|-----------------------------------------------|--------------------------|----------------|
| White* 1986             | ALPO<br>Chicken or lamb<br>HC chicken & rice | 26                                   | 6 (Lamb diet/<br>OK on HC lamb & rice) | 2 weeks each diet                             | Recurrence c/s           |                |
| Sousa et al 2000        | Purina HA                                    | 24                                   | 2                                      | 1 week                                        | ↑↑ pruritus              |                |
| Wagner & Horvath 1999   | Pedigree Canine selected protein 3           | 16                                   | 1                                      | 2 weeks                                       | ↑↑ pruritus              |                |
| Leistra et al 2001      | Chicken<br>Venison<br>Catfish (Waltham)      | 40                                   | 19<br>21<br>34                         | 3 weeks                                       |                          | 1-21 days      |
| Rosser 2001             | Exclude                                      | 19                                   | 1                                      | 30 days                                       |                          |                |
| Roudebush & Schick 1995 | Prescription Diet d/d Lamb & rice (Hills)    | 20                                   | 3                                      | 18-60 days                                    | Return of Clinical signs |                |
| Tapp et al. 2002        | Eukanuba Response FP                         | 8                                    | 4                                      | NR                                            | ↑↑ Pruritus              | 1 day- 6 weeks |
| Beale & Laflamme 2001   | Purina HA                                    | 10<br>Known Sensitivity to soy/ corn |                                        | 2 weeks each diet (positive/ negative & test) | ↓ Pruritus               |                |

Table 2. Diets trialled in dogs with known AFR

Exclude, DVM pharmaceuticals: pinto beans, oats, hydrolyzed casein & chicken liver.

Purina Veterinary Diets HA-formula, Nestle Purina Co: hydrolysed soy & cornstarch

Pedigree Canine Selected Protein 3: capelin & tapioca

Waltham veterinary Diet, Pedigree selected protein: chicken and rice OR venison and rice OR catfish and rice

Eukanuba Response FP:

Prescription diet canine d/d canned. Lamb & rice. Hills

## Diagnostic testing

The gold standard against which diagnostic tests are measured is a LADT followed by demonstrating that on challenge with previously fed foods, the clinical signs recur.

In most cases the clinician/ investigator has relied on the client's observational skills to confirm a positive challenge. The time to relapse is not always specified, nor is the specific sampling time.

### 1. Prick testing

- One of the earliest (and most entertaining) reports by Schnelle in 1933 describes two dogs which were prick tested with various commercial allergens. They were found to be positive to salmon and subsequently developed clinical signs when fed the offending protein.

#### Intradermal testing

- b. One hundred dogs aged > 6 months were skin tested for environmental and food allergens; 48/100 reacted positively to food antigens. Twenty eight of these dogs underwent a LADT for 3 weeks. Three were confirmed with ARF. Additionally 35 dogs negative on serology underwent a LADT, six of these dogs were subsequently confirmed with AFR.(Kunkle & Horner 1992)
  - c. Dogs selected for LB (see 5 below) also underwent intradermal testing; in 2/11 cases positive reactions were seen to beef on IDT and oral challenge.(Ishida et al 2004)
2. Allergen specific serology
    - a. Serum from eight affected dogs confirmed by LADT and challenge was compared with serum from eight non-affected controls, one cat and one horse using a monoclonal ELISA for allergen specific IgE. Mild positives were registered in three of the canine controls. The sampling time post challenge was not specified. (Mueller & Tsohalis 1998)
  3. PK & oral PK
    - a. Serum from ten dogs with AFR confirmed with LADT and challenge was tested using the PK and oral PK test. An antigen specific response was not detected. (Hillier & Kunkle 1994)
  4. Lymphocyte blastogenesis

Eleven dogs with adverse food reaction confirmed by a LADT of up to eight weeks were selected, age > 1 year. Seven manifested clinical signs of pruritus and four had gastrointestinal signs. Six unaffected controls were employed. Lymphocyte blastogenesis was performed on two occasions. Once during elimination and once during provocation 1-21 days post challenge. In 9/11 affected dogs the lymphocyte blastogenesis was increased with antigen provocation as compared with the normal controls.

#### Conclusion

Both the pathogenesis and clinical presentation are poorly defined in the dog with AFR and the incidence of dogs with pure food allergy v food intolerance is currently unknown. Based on this literature review and to further investigate canine AFR the author offers the following suggestions:

1. Dogs undergoing LADT for diagnosis of AFR should be selected with similar clinical signs and a similar age of onset of disease.
2. Response to a LADT should be monitored with an objective scoring system
3. Dogs with concurrent hypersensitivities should be excluded thus the end point is complete resolution of clinical signs.
4. The response to dietary challenge should be monitored by a clinically trained observer
5. The placebo effect should not be underestimated and consideration should be given to performance of a random placebo controlled trial.
6. The timing of measurements of allergen specific IgE should relate to allergen exposure.

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## NOTES

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# EVIDENCE-BASED TREATMENT OF CANINE ATOPIC DERMATITIS: 2010 PRACTICE GUIDELINES

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*Note: this is a short summary of an extensive article that can be downloaded with open access at:  
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<http://onlinelibrary.wiley.com/doi/10.1111/j.1365-3164.2010.00889.x/supinfo>*

Atopic dermatitis (AD) is a common chronic relapsing pruritic skin disease of dogs for which treatment has varied over time and geographical location. Recent high quality randomized controlled trials and systematic reviews have established which drugs are likely to offer consistent benefit. In 2010, the International Task Force for Canine AD published guidelines recommending a multi-faceted approach to treat dogs with AD. Treatment recommendations vary depending if one is dealing with acute flares or chronic AD, and whether skin lesions are localized or extensive.

## **TREATMENT OF ACUTE FLARES OF ATOPIC DERMATITIS**

### **IDENTIFICATION AND AVOIDANCE OF FLARE FACTORS**

#### **IDENTIFICATION AND REMOVAL OF ALLERGENIC CAUSES OF FLARES**

When an exacerbation of signs occurs in a dog that previously had a disease in remission, one must look for, and eliminate if at all feasible, the cause of such flares. Currently recognized sources of flares of canine AD include fleas, food and environmental (e.g. house dust mites, pollens) allergens.

#### **EVALUATION OF USE OF ANTIMICROBIAL THERAPY**

Skin and ear infections are common reasons why lesions and pruritus acutely worsen in dogs with AD. If bacterial or yeast infections are identified with some combination of clinical signs, cytology and/or culture, antimicrobial therapy is indicated, normally using topical with or without oral medications. The latter are used if infected lesions are severe or extensive.

## **IMPROVEMENT OF SKIN AND COAT HYGIENE AND CARE**

### **BATHING WITH A NON-IRRITATING SHAMPOO**

Bathing dogs with AD might reduce their pruritus (itch) manifestations. This benefit appears to lie in the mechanical action of washing the pet. Outside of a lipid-containing shampoo (Allermyl, Virbac), there is currently no evidence of benefit of other shampoos or conditioners containing ingredients such as oatmeal, pramoxine, antihistamine, lipids or glucocorticoids.

## **REDUCTION OF PRURITUS AND SKIN LESIONS WITH PHARMACOLOGICAL AGENTS**

### **SHORT-TERM TREATMENT WITH A TOPICAL GLUCOCORTICOID**

To reduce skin lesions and pruritus of canine AD, there is evidence for the high efficacy of two medium potency glucocorticoid sprays: triamcinolone (Genesis, Virbac) and hydrocortisone aceponate (Cortavance, Virbac). These sprays are especially suitable for localized skin lesions and for short durations. Clinicians must tailor the frequency and duration of application to the severity of clinical signs. Caution is advised with long-term use, as adverse effects, such as skin thinning, are likely to occur.

### **SHORT COURSE OF ORAL GLUCOCORTICOIDS**

If signs are too severe or extensive to be controlled with topical formulations, then oral glucocorticoids are recommended. Either prednisone, prednisolone or methylprednisolone can be given at 0.5 mg/kg once to twice

daily until clinical remission occurs. Side effects of oral glucocorticoids are usually proportional to drug potency, dosage and duration of administration.

***INTERVENTIONS LIKELY TO BE OF LITTLE OR NO BENEFIT TO TREAT ACUTE FLARES OF CANINE AD:***

***Antihistamines:*** When examined as a group, there is no conclusive evidence of efficacy of oral type-1 antihistamines for treatment of active AD in dogs.

***Essential Fatty Acid Supplements:*** As their mode of action necessitates several weeks of treatment, essential fatty acids (EFA) are unlikely to be of any benefit for acute flares of AD in dogs.

***Tacrolimus and Ciclosporin:*** Because of their slow onset of treatment effect, topical tacrolimus and oral ciclosporin are unlikely to offer any benefit for treatment of acute flares of canine AD.

**TREATMENT OPTIONS FOR CHRONIC CANINE AD**

***IDENTIFICATION AND AVOIDANCE OF FLARE FACTORS***

***PERFORMANCE OF DIETARY RESTRICTION-PROVOCATION TRIALS IN DOGS WITH NONSEASONAL AD***

Food allergens can cause flares of AD in dogs hypersensitive to such allergens. As a result, one or more restriction-provocation dietary trials (e.g. 'elimination diets') must be performed in all dogs with nonseasonal AD to determine whether food allergens contribute to clinical signs in these patients. Normally, dietary changes should be carried out for six to ten weeks using either commercial or homemade diets employing a low number of novel or hydrolyzed ingredients. At this time, there is no clear evidence of a superior benefit of hydrolysate-based compared to non-hydrolyzed commercial diets, or of homemade over commercial diets. In theory, the main value of performing trials with homemade diets is if hypersensitivity to a minor component of a commercial diet (colorant, preservative, etc.) is suspected, but cutaneous hypersensitivity to additives has not yet been reported in dogs.

***IMPLEMENTATION OF AN EFFECTIVE FLEA CONTROL REGIMEN***

There is evidence that the atopic status predisposes dogs to develop hypersensitivity to flea saliva if exposed repeatedly to flea bites. As a result, where flea infestation is endemic, all dogs with AD should be treated with year-round flea adulticides combined with relevant environmental measures.

***PERFORMANCE OF ALLERGEN-SPECIFIC INTRADERMAL AND/OR IGE SEROLOGICAL TESTS TO IDENTIFY POSSIBLE ENVIRONMENTAL ALLERGEN FLARE FACTORS***

Environmental allergens, such as house dust mites, have been shown to cause flares of AD in dogs hypersensitive to these allergens. The performance of allergen-specific intradermal testing (IDT) and/or IgE serological tests is helpful to identify hypersensitivity to environmental allergens in dogs with AD. Importantly, positive immediate IDT reactions and IgE serologies to environmental allergens are also common in dogs without signs of AD. As a result, these tests cannot be used to differentiate dogs with AD from normal dogs. Serological and intradermal tests to determine hypersensitivity to food allergens do not reliably predict food allergies, and therefore they cannot be recommended.

***IMPLEMENTATION OF HOUSE DUST MITE CONTROL MEASURES***

*Dermatophagoides* house dust mite proteins are the most common allergens in dogs with AD. Household dust mite control measures 'theoretically' should be effective for mite-allergic patients. However, even when specific products have been shown to measurably decrease dust mite allergen in the environment, this might not necessarily lead to an improvement in clinical signs in hypersensitive individuals. Nevertheless, if mite avoidance measures were to be attempted, it would seem logical to restrict this intervention to dogs sensitized to house dust mites alone, and to use a combination of measures that might include acaricides, impermeable pet mattress covers, and frequent and thorough pet mattress and environment washing and vacuuming. A benefit, if any, is likely to take some months to occur due to the long persistence of mite allergens in the environment.

### ***EVALUATION OF USE OF ANTIMICROBIAL THERAPY***

The skin and ears of dogs with AD are commonly infected or colonized with *Staphylococci* and *Malassezia* species. It is suspected that these microorganisms might contribute to the severity of AD outside of “classical” superficial infections (e.g. bacterial folliculitis). Veterinarians are encouraged to: 1) identify skin lesions suggesting microbial colonization (e.g. erythema, oedema, scaling, greasiness) at particular sites, including the ears, 2) document the presence of bacteria/yeast at these lesional sites, 3) implement specific antibacterial/antifungal interventions, 4) using cytology, observe the disappearance of organisms from previously positive sites following antimicrobial interventions, and 5) document the reduction/disappearance of skin lesions at the previous sites following antimicrobial interventions. The systematic prescription of antibiotics and antifungal drugs to every dog with AD is not recommended, however, as such routine use of antimicrobial drugs is likely to increase the prevalence of drug-resistant microbes.

### ***Investigation of the relevance of other flare factors***

In human patients with AD, environmental (e.g. low humidity, clothing, detergents) and psychological factors (e.g. stress) are known contributors to the severity of clinical signs of AD. At this time, there is insufficient evidence on the role of such factors as a cause of flares of AD in dogs.

### ***Improvement of skin and coat hygiene and care***

#### ***Bathing with a non-irritating shampoo***

Weekly bathing with a mild non-irritating shampoo and lukewarm water is likely to be beneficial for a direct soothing effect to the skin, the physical removal of surface allergens and microbes and an increase in skin hydration. At this time, there is no evidence of superiority of any particular shampoo or protocol to achieve these goals. If the skin is greasy and scaly, antiseborrheic shampoos are indicated. If infections are deemed to contribute to clinical signs, antiseptic shampoos are preferred. In some cases, moisturizers might alleviate any skin dryness that would occur after the baths.

### ***DIETARY SUPPLEMENTATION WITH EFA***

In normal dogs, dietary supplementation with EFA, or the feeding of EFA-rich diets (especially those rich in the omega-6 EFA linoleic acid) usually results in improvement in coat quality and gloss. Two diets have had this improvement documented in good quality clinical trials: Specific Skin & Joint Support (Dechra Veterinary Products) or the Hill’s Prescription Diet Canine d/d Salmon & Rice. Not all EFA-rich diets appear to have such coat improvement effect. At this time, there is no evidence of superiority of any particular EFA combination, dosage, ratio or formulation (including enriched diets) to improve skin and coat quality in dogs with AD, but, in general, EFA-enriched diets provide higher amounts of EFA than oral supplements. The benefit of EFA, if any, might not be seen before two months of supplementation.

### ***TOPICAL LIPID FORMULATIONS***

At this time, there is insufficient evidence supporting the use of topical formulations containing EFA, essential oils, or complex lipid mixtures for improvement of coat quality, barrier function or any other clinically relevant benefit in dogs with AD.

### ***OTHER DIETARY SUPPLEMENTS***

Several nutritional supplements (e.g. pantothenate, choline, nicotinamide, histidine and inositol) have been shown to increase the production of skin lipids *in vitro* and to decrease transepidermal water loss *in vivo* in healthy dogs. Additional studies are needed to confirm the clinical benefit of diets containing these supplements in dogs with AD.

### ***REDUCTION OF PRURITUS AND SKIN LESIONS WITH PHARMACOLOGICAL AGENTS***

#### ***TREATMENT WITH TOPICAL GLUCOCORTICOIDS OR TACROLIMUS***

As discussed above, there is good evidence supporting the efficacy of topical glucocorticoids for treatment of AD in dogs. Clinicians must tailor the frequency and duration of application of topical glucocorticoids to the severity

of clinical signs. Such formulations are best suited for focal (e.g. foot) or multifocal lesions and for relatively short durations (e.g. less than two months).

The most common and important adverse events following the prolonged application of a potent topical glucocorticoid on the same area are thinning of the skin (cutaneous atrophy), black heads (comedones) and superficial hair follicle cysts (milia). The risk is lower with intermittent application of topical glucocorticoids.

As an alternative to topical glucocorticoids, 0.1% tacrolimus ointment (Protopic, Astellas) has been shown to be effective, especially in dogs with localized AD. The efficacy of tacrolimus ointment appears highest when used twice daily for one week with later reduced frequency of application as needed to control signs. Signs suggesting mild irritation might follow the application of tacrolimus.

#### *TREATMENT WITH ORAL GLUCOCORTICOIDS OR CICLOSPORIN*

There is strong evidence of the efficacy of oral glucocorticoids and ciclosporin for treatment of AD in dogs. Such oral medications are especially suited for dogs with generalized AD, and when other flare factors have been identified and eliminated. The onset of clinical benefit arises earlier with glucocorticoids than with ciclosporin.

As discussed above, oral glucocorticoids (e.g. prednisone, prednisolone, methylprednisolone) should be started at approximately 0.5 mg/kg once to twice daily, and then reduced, as signs decrease, to the lowest dose and frequency (e.g. twice daily to once daily to every other day) needed to maintain good quality of life, control of signs and minimal side effects. Side effects of oral glucocorticoids (e.g. increased appetite, drinking and urination, predisposition to urinary tract infections) are common and normally proportional to dosage and duration of administration. At this time, because of the risk for adverse effects, the use of long-acting injectable glucocorticoids is not recommended unless there is an inability to treat the patient orally.

In an attempt to reduce the dose of oral glucocorticoids needed to control clinical signs of AD, veterinarians are encouraged to investigate medications or supplements proven to have a steroid-sparing effect, for example, the glucocorticoid-antihistamine combination Temaril-P (Pfizer), the EFA-combination Viacutan Plus (Boehringer Ingelheim) and the Chinese herbal supplement Phytopica (Intervet-Schering).

Modified ciclosporin (Atopica, Novartis) should be started at a dosage of 5 mg/kg once daily and continued at this dosage until a halving or a satisfactory decrease of severity of signs is achieved. After this improvement is reached, the dose should be reduced by either increasing dosage intervals (e.g. going from every day to every other day) or by decreasing the daily dose by half. After a further reduction of signs exceeding approximately 75%, the administration could be reduced to twice weekly or a 75% reduction of the original daily dose. After beginning ciclosporin administration, the onset of satisfactory clinical benefit normally cannot be expected before four to six weeks. To increase the speed of clinical sign improvement, the administration of a short course of oral glucocorticoids – as described above – during the first two weeks of ciclosporin administration might be beneficial. Minor adverse events (e.g. vomiting, diarrhoea) are common after initiating ciclosporin therapy; most improve spontaneously upon further administration of this drug.

#### *TREATMENT WITH SUBCUTANEOUS INTERFERONS*

There are studies providing evidence of the efficacy of injections of recombinant canine gamma-interferon (Interdog, Toray) to treat dogs with AD in Japan. Suggested effective dosages are 5,000 to 10,000 units/kg, subcutaneously, three times weekly for four weeks then once weekly. Side effects are minimal. Similarly, recombinant feline omega interferon (Virbagen Omega, Virbac) also appears effective to treat dogs with AD in Europe. Suggested doses of one to five million units three times weekly for four weeks and then every month are well tolerated.

### ***INTERVENTIONS LIKELY TO BE OF LITTLE OR NO BENEFIT TO TREAT CHRONIC CANINE AD:***

Results from clinical trials suggest that, as a group, first (i.e. sedating) and second (i.e. lower sedation) generation oral type 1 antihistamines are unlikely to be beneficial in dogs with chronic AD skin lesions. If veterinarians wish to use type 1 antihistamines, they should limit their prescription to those drugs with demonstrable antihistamine effect in dogs (e.g. hydroxyzine at 2 mg/kg twice daily or cetirizine 0.5-1.0 mg/kg once daily). Finally, antihistamines should be given as preventatives, that is every single day at the recommended dosage, to keep blocking histamine receptors before histamine is released. The main side effect of most antihistamines is sedation.

A systematic review of clinical trials provides evidence that EFA supplements, EFA enriched diets and nutritional or herbal supplements are unlikely to provide meaningful benefit if given alone for relief of inflammation and/or pruritus. As discussed above, EFA might be useful to improve coat quality and ameliorate dry skin, but, at this time, there is no evidence of superiority of any particular EFA combination, dosage, ratio or formulation (including enriched diets) to achieve skin barrier, coat quality or anti-allergic effect.

There is some evidence of anti-allergic efficacy of oral pentoxifylline, misoprostol and tepoxalin, but because of their modest benefit, potentially high costs and adverse effects, these medications should probably not be used as first line medications to treat dogs with AD.

Finally, there is some evidence of very low, or complete lack of efficacy of leukotriene inhibitors, dextromethorphan or capsaicin to treat dogs with AD. Consequently, these drugs should not be used to treat dogs with this disease.

### ***IMPLEMENT STRATEGIES TO PREVENT RECURRENCE OF SIGNS***

#### ***AVOIDANCE OF FLARE FACTORS***

Avoidance of known flare factors is the strategy most optimal to prevent recurrence of signs in patients with AD. As discussed in the sections above, the maintenance of the dog on a diet not containing ingredients to which it is hypersensitive, the implementation of an effective flea control and a reduction of contact with provocative environmental or microbial allergens would be ideal, wherever and whenever possible.

#### ***IMPLEMENTATION OF PROACTIVE (PREVENTIVE) PHARMACOTHERAPY***

In humans with AD, there is evidence of high benefit, low cost and low risk of proactive intermittent applications of topical glucocorticoids and tacrolimus to skin areas repeatedly affected during flares of AD. Whether or not a similar strategy would be equally effective in dogs with AD has not been established at this time, but because of the possible benefit, low risk and low cost, such interventions are worth considering in dogs with recurrent moderate or severe AD.

#### ***IMPLEMENTATION OF ALLERGEN-SPECIFIC IMMUNOTHERAPY***

Allergen-specific immunotherapy (ASIT) is the practice of administering gradually increasing quantities of an allergen extract to an allergic subject to ameliorate the symptoms associated with subsequent exposure to the causative allergen. Subcutaneous ASIT appeared effective and safe to reduce signs of AD in dogs. It should be considered in any dog in which intradermal test or IgE serology have permitted the identification of allergens likely to contribute to the disease and in whom allergen contact is unavoidable. The dog's owners should be able to afford the time, expense and technical aspects of this regimen. In addition, when symptomatic anti-inflammatory therapy is ineffective, or associated with unacceptable or potentially unacceptable side effects (e.g. glucocorticoids), or is impractical to maintain for an extended period of time, then ASIT is indicated, even in dogs with seasonal disease of short duration. Finally, due to its unique mode of action, ASIT is the only intervention that has the potential to prevent the development of signs and alter the long-term course of the disease.

It is expected that between approximately 50 and 80% of dogs with AD that have been treated with ASIT for six to twelve months will exhibit an improvement in signs and/or a decrease in anti-inflammatory or antipruritic medication use. At this time, there appears to exist no clear advantage of a particular ASIT protocol (traditional, rush or low-dose). Most importantly, injection frequencies and amounts injected must be tailored to each patient depending upon the clinical improvement observed and the presence of adverse events (e.g. increases in pruritus after each injection). Because of the delay in ASIT effect, anti-inflammatory drugs should be given temporarily, as needed to maintain good quality of life until ASIT might offer clinical benefit. Immunotherapy must be continued for at least one year before dismissing it as ineffective.

## NOTES

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# PROBIOTICS: BACKGROUND, MECHANISMS OF EFFECT, AND CLINICAL POSSIBILITIES

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## Introduction

The mammalian intestinal tract contains a complex, dynamic, and diverse society of pathogenic and nonpathogenic bacteria. Researchers have estimated that the human body contains  $10^{14}$  cells, only 10% of which are not bacteria, and belong to the human body proper (Savage, 1977). There has been a plethora of research focusing on the mechanisms by which pathogenic bacteria influence intestinal function and induce disease; however, recent attention has focused on the indigenous non-pathogenic microorganisms and the ways in which they may benefit the host. Documentation of the health benefits of bacteria in food dates back to as early as the Persian version of the Old Testament (Genesis 18:8), and Plinius, a Roman historian in 76 BC, recommended the use of fermented milk products for the treatment of gastroenteritis (Bottazi). One-hundred years ago, the Nobel Prize-winning Russian scientist, Elie Metchnikoff, suggested that the ingestion of lactobacillus-containing yogurt decreased the number of toxin-producing bacteria in the intestine, contributing to the longevity of Bulgarian peasants (Metchnikoff 1907). These observations led to the concept of a “probiotic”, derived from the Greek, meaning “for life.” The term “*probiotic*” was first used in 1965 to define “substances secreted by one microorganism which stimulates the growth of another” and was thus contrasted with the term *antibiotic* (Lilly, 1965). The meaning of the word has subsequently evolved to apply to those bacteria that “contribute to intestinal balance.” The current and more complete definition of a probiotic refers to “a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora in a compartment of the host and by that exert beneficial health effects on the host.” (Schrezenenmeir 2001).

Different strains of probiotic bacteria may exert different effects based on specific capabilities and enzymatic activities, even within one species (Ouwehand, 1999; Bernet, 1993). Different microorganisms express habitat preferences that may differ in various host species. Four microhabitats in the gastrointestinal tract were outlined by Freter (1992) as follows: 1) the surface of epithelium cells; 2) the crypts of the ileum, cecum, and colon; 3) the mucus gel that overlays the epithelium; and 4) the lumen of the intestine. The luminal content of bacteria depends greatly on bowel transit, resulting in a relatively low microbial density in the small bowel. It should be emphasized that a proven probiotic effect found in one strain or species cannot be transferred to other strains or species because of differences in strain characteristics and habitat preferences.

Because of these multiple mechanisms of action, many different probiotics have potential applications to various diseases. Those in most widespread use, which have undergone the most clinical testing in humans and animals, include *Lactobacillus* species (such as *L acidophilus*, *L rhamnosus*, *L bulgaricus*, *L reuteri*, and *L casei*); *Bifidobacterium* species; and *Saccharomyces boulardii*, which is a nonpathogenic yeast. In dogs and cats, *Enterococcus faecium* has also received a lot of attention in clinical use in Europe and the US. Nevertheless, despite the explosion of interest and publications on probiotics in recent years, the clinical application of probiotics has been limited by the paucity of well-designed and mechanistically based laboratory, translational, and clinical studies.

## Probiotics: Regulatory Aspects and Safety

The guidelines for what is required for a product to be called a probiotic were published by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), and require that strains be designated individually, speciated appropriately, and retain a viable count at the end of their shelf life in the designated product formulation that confers a proven clinical end-point (FAO/WHO 2002). The fact that some products continue to be of dubious quality and carry unsupported health claims, complicates the process. This problem is compounded by the diverse categories that encompass probiotic products, including: food, functional food, novel food, natural remedy (Denmark, Sweden and Finland), natural health product (Canada), dietetic food (Italy), dietary supplement (USA), and biotherapeutic and pharmaceuticals (probiotic pharmaceuticals are available in Canada, China, and a variety of European countries).



The definition of a probiotic requires that the term only be applied to live microbes having a substantiated beneficial effect. Thus, microbes administered alive are considered probiotics regardless of their ability to survive intestinal transit. Although a preparation of non-viable bacteria may mediate a physiologic benefit, they are not considered to be “probiotics” under the present definition. Furthermore, any strains that do not confer clinically established physiological effects should not be referred to as probiotics. *In vitro* testing to establish mechanisms of action are insufficient substantiation for the use of the term, “probiotic.” The basis for a microbe being termed a probiotic should be proven efficacy and safety under the recommended conditions of use, with considerations given to target population, route of administration, and dose applied (FAO/WHO 2002).

Despite the prolonged marketing of “probiotic” products, there is little or no enforced worldwide regulation regarding labeling for quality or efficacy. A relatively large number of products are mislabeled based on inaccurate use of nomenclature for genus and species, inaccurate cell count or unsubstantiated structure/function statements continue to be sold worldwide (Weese 2001).

Probiotic preparations labeled for use in dogs or cats are classified as nutritional supplements, not pharmaceutical products. As a result, they are not highly regulated, and specific product labeling and demonstration of efficacy are not required. This important point is best illustrated by a recent study by Weese et al. in which nineteen commercially available canine and feline diets purporting to contain probiotics were evaluated bacteriologically (Weese 2003). Quantitative bacterial cultures were performed on all products and the labeling claim of each product was compared to the qualitative and quantitative culture results. None of the products contained all of the claimed organisms, while 1 or more of the listed contents were isolated from 10 of 19 (53%) products, and five (26%) diets did not contain any relevant growth. The diets that were tested contained between 0 and  $1.8 \times 10^5$  CFU/g. Of equal concern, the question of what constitutes a minimal effective dose of a probiotic has yet to be defined.

### **Antibiotic Resistance and Probiotics:**

Antibiotic resistance screening has shown that the spontaneous mutation rate to antibiotic resistance among *Lactobacilli* can be quite high in the order of  $2 \times 10^5$ , depending on the strain (Curragh, 1992). Several animal isolates of *Lactobacillus acidophilus* and *Lactobacillus reuteri* were tested for antibiotic resistance and all 16 *L. reuteri* strains were resistant to vancomycin and polymyxin B irrespective of their source, while only four of thirty *L. acidophilus* strains were vancomycin resistant and seven were chloramphenicol resistant (Sarra 1982). Antibiotic resistance plasmids from lactobacilli have been detected in a number of studies. Although enterococci are normal inhabitants of the gastrointestinal tract and are widely used as both human and animal probiotics, *in vivo* conjugative transfer of antibiotic resistance plasmids from *L. reuteri* to *E. faecalis* has been demonstrated in germ-free mice (Morelli 1988). In most cases, antibiotic resistance to LAB is not of the transmissible type, but represents an intrinsic species or genus specific characteristic of the organism. Knowledge of the ability of a proposed probiotic strain to act as a donor of conjugative antibiotic resistance genes is a prudent precaution. Although the enterococcal transmissible vancomycin resistance poses an important issue, to date there is no evidence of this occurring in clinical cases.

## **MECHANISMS OF PROBIOTIC ACTION**

### **A) Probiotics block intestinal bacterial effects**

Probiotics have been identified to mediate maintenance of the gastrointestinal microbial balance via two mechanisms: production of antibacterial substances, such as bacteriocins (e.g. lantibiotics) and acids (e.g. acetic, lactic, and propionic), and competitive inhibition of pathogen and toxin adherence to the intestinal epithelium. Several strains of *Lactobacilli* and *Bifidobacteria* are able to decrease adhesion of both pathogens and their toxins to the intestinal epithelium, and they can displace pathogenic bacteria even if the pathogens have attached to intestinal epithelial cells prior to probiotic treatment (Collado 2007, Candera 2005). One of the mechanisms underlying pathogenic bacteria binding to intestinal epithelial cells is through the interaction between bacterial lectins and carbohydrate moieties of glycoconjugate receptor molecules on the cell surface (Mukai 2004, Sun 2007).

## **B) Probiotics regulate mucosal immune responses**

Both *in vitro* and *in vivo* studies show effects of probiotics on host immune functions, including upregulation of immune function that may improve the ability to fight infections or inhibit tumor formation; downregulation of immune function that may prevent the onset of allergy or intestinal inflammation. The following is a brief overview of the many possible ways individual probiotic species may have an effect on the animal's mucosal (and ultimately, systemic immune response)

- **Enhancing Host Innate Immunity**

Probiotics have the potential to stimulate innate immune responses against microorganisms and dietary antigens newly encountered by the host through several mechanisms. Intestinal dendritic cells can retain commensal bacteria by selectively activating B lymphocytes to produce IgA to reduce mucosal penetration by bacteria. The dendritic cells carrying commensals are restricted to the intestinal mucosal lymphoid tissues, and thus avoid potential systemic immune responses (MacPherson 2004).

- **Modulation of Pathogen-induced Inflammatory Responses**

The host innate defenses must modulate responses appropriate to the level of threat provided by a given pathogen. If the response is too weak, the infection may not be cleared, leaving the host susceptible to systemic infection. However, if it is too strong the result may be excess tissue damage. A mechanism of probiotic protection from pathogen induced injury and inflammation is modulating the balance of pro- and antiinflammatory cytokine production.

- **Increasing Antiinflammatory Cytokine Production**

Probiotics can induce dendritic cells to produce antiinflammatory cytokines, including IL-10, which suppress the Th1 response (Hart AL, 2004). However, the role of IL-10 production in probiotic prevention of Th1 responses by probiotics is controversial, and may be through both IL-10- dependent and –independent mechanisms.

- **Suppressing Proinflammatory Cytokine Production**

Probiotics such as LGG have been shown to inhibit lipopolysaccharide (LPS) and *Helicobacter pylori*-stimulated TNF production by murine macrophages (Penna 2003). In addition, LGG-conditioned cell culture media decreases TNF production in macrophages, indicating that soluble molecules derived from LGG exert this immunoregulatory role (Penna 2003).

- **Upregulation of Host Immune Responses to Defend Against Infection**

Probiotics and commensal microflora may regulate a balance between pro- and antiinflammatory mucosal responses leading to intestinal homeostasis. Probiotics facilitate this important function by stimulation of host immunological functions, including Th1 responses through dendritic cell-directed T cell activation. During colonization of mice with *B. fragilis*, dendritic cells take up and retain a bacterial polysaccharide which promotes maturation of dendritic cells, Th1-type cytokine production including IL-4, IL-12, and IFN- $\gamma$ , and subsequent CD4 + T cell expansion (Mazmanian 2005).

- **Regulation of Immune Responses by Probiotic DNA**

There have been a number of intriguing studies documenting the beneficial immunomodulatory properties of probiotic DNA in people and murine models. DNA isolated from the probiotic VSL#3 mixture decreases LPS-activated IL-8 production and TNF and IFN- release *in vivo* and *in vitro* (Jijon 2004).

- **Differential Activation of TLRs by Probiotics in Immune Cells**

Different probiotic bacteria stimulate distinct TLRs on host cells, an essential consideration in designing any therapeutic trials. Probiotic bacteria possess molecular recognition patterns similar to pathogenic bacteria; however, the probiotic organisms do not normally initiate pathogenic inflammatory responses. It appears that probiotics exert both up- and downregulatory effects on immune responses, and TLR-regulated signaling pathways appear to be one of the mechanisms for these immunoregulatory actions. The probiotic *E. coli* Nissle 1917 express increased levels of both TLR2 and TLR4 (Grabig 2006) whereas the probiotic VSL#3 mixture mediates its immunostimulatory response via TLR9 signaling (Rachmilewitz 2004). There is clearly a need to define the mechanism(s) for observed differences among the signals induced by probiotics and pathogens, which use similar receptors to induce divergent responses.

### **C) Probiotics regulate intestinal epithelial cell functions**

Substantial evidence indicates that probiotic bacteria stimulate intestinal epithelial cell responses, including restitution of damaged epithelial barrier (Zyrek 2007), production of antibacterial substances and cell-protective proteins (Tao 2006), and prevention of cytokine-induced intestinal epithelial cell apoptosis (Yan 2002). Many of these responses result from probiotic stimulation of specific intracellular signaling pathways in the intestinal epithelial cells.

### **CURRENT APPLICATIONS FOR PROBIOTICS IN HUMANS**

One important characteristic of probiotics is their ability to suppress the proliferation and virulence of pathogenic organisms, and this is an increasingly well-documented role of probiotic bacteria in the gastrointestinal tract and genitourinary tract. However, it is becoming increasingly clear that probiotic microbiota have direct effects on human physiology and immunity, including allergic disease (e.g. asthma, hay fever), autoimmune diseases (e.g. multiple sclerosis and type I diabetes), diseases of the oral cavity (e.g. periodontal disease and caries), and the nervous system (e.g. autism and depression) (Spinler and Verslovic, 2008).

#### **Probiotics in Allergy and Autoimmune Disease**

There has been an increasing incidence of allergic and autoimmune diseases (i.e. type I diabetes, multiple sclerosis) in humans from western countries (Bach 2002). There are many potential reasons for this, but a common theory is the “hygiene” hypothesis that attributes the failure of immunoregulation caused by decreased exposure to organisms that were a part of their evolutionary history. The hygiene hypothesis is based on the concept that exposure to these organisms is important because they drive the development of immunoregulatory mechanisms of tolerance. Interestingly, the validity of these arguments is supported by clinical trials and experimental models in which parasites and microorganisms that are depleted from the environment in Westernised countries have been shown to treat allergy (Wilson 2005, Ricklin-Gutzwiller 2007), autoimmunity (Calcinaro 2005, Zacccone 2003), or intestinal inflammation (Di Giacinto 2005).

#### **Probiotic Therapy in Dogs**

To date, only a relatively small number of studies have been published evaluating the effects of probiotics in dogs, and most of these have been focused on the intestinal microflora in apparently healthy dogs. Specifically, probiotic strains of human or canine origin (*Lactobacilli*, *Bifidobacter* and *Enterococcus*) were used in healthy adult dogs to assess effects on intestinal microbial populations, reduction of specific pathogens in feces, and immunomodulation (Strompfova 2004, Sauter 2006, Perelmuter 2008, Pascher 2008, Vahjen and Manner 2003, Swanson 2002, Biagi 2007). In many of these studies, the effect of probiotics added to the food in healthy dogs had an equivocal effect on fecal microflora and pathogens (Vahjen and Manner 2003, Baillon 2004). Further, it is important to note that most of these studies were not randomized, controlled trials, and the strains of probiotic varied from study to study, making interpretation of findings more challenging. In addition, many studies focused on fecal isolation and quantitative cultures of putative pathogenic bacteria such as *C. perfringens*, rather than evaluating more meaningful end-points such as shifts in the microbial flora, mucosal immunopathology, and alterations in intestinal integrity. Only two studies addressing the role of probiotics in management of dietary sensitivity and food responsive diarrhea have been published to date, with overall positive results (Pascher 2008, Sauter 2006). Only one of these studies was a randomized, placebo-controlled clinical trial (Sauter), and the results of that study, while clinically positive because all of the dogs on the study improved when they were placed on the elimination diet, showed no specific changes in the inflammatory cytokine patterns of the dogs or a specific benefit of the probiotic (Sauter 2006). The immunomodulatory effects of *Enterococcus faecium* SF68 have been studied in dogs, and the probiotic was associated with increased fecal IgA concentrations and increased vaccine specific circulating IgG and IgA concentrations (Benyacoub 2003). While increased immune globulins may suggest enhanced immune response, the clinical relevance of this finding is not known. Additional studies are warranted in dogs to further assess the immunomodulatory effects of probiotics, and to evaluate their safety. The latter issue is particularly important given the recent finding of increased intestinal adhesion of *Campylobacter jejuni* in an *in vitro* model of canine intestinal mucus following incubation with *Enterococcus faecium* (Rinkinen 2003). It should be noted that this *E. faecium* strain is different from the *E. faecium* SF68 strain that is commercially available and to date, there is no clinical or anecdotal evidence of *Campylobacter*-

associated diarrhea in dogs. Despite the paucity of prospective, randomized placebo-controlled clinical trials in dogs, tremendous interest has been shown among commercial pet-food companies who are marketing probiotics for use in dogs or cats. Unfortunately, most of the evidence surrounding the use of probiotics in puppies or adult dogs with stress colitis or antibiotic-responsive diarrhea is anecdotal, with no prospective, randomized, placebo-controlled studies in these disorders published to date.

### **Probiotic Therapy in Cats**

Unfortunately, there is a dearth of published information pertaining to probiotic use in cats, and there are no clinical studies reporting a beneficial effect of probiotic therapy for any feline disease (Weese 2008). One study evaluating the effect of dietary supplementation with the probiotic strain of *Lactobacillus acidophilus* DSM 13241 ( $2 \times 10^8$  CFU/d for 4.5 weeks) administered in 15 healthy adult cats demonstrated the recovery of the probiotic from the feces of the cats in association with a significant reduction in *Clostridium* spp. and *Enterococcus faecalis* (Marshall-Jones 2006). However, the immunomodulatory effects were reported based on decreased lymphocyte and increased eosinophil populations, and increased activities of peripheral blood phagocytes. The relevance of these findings is unclear as this study was not a randomized trial and the changes reported in the populations of peripheral blood cells cannot be extrapolated into evidence of systemic health benefits. Evaluation of the effect of supplementation with *Enterococcus faecium* strain SF68 on immune function responses following administration of a multivalent vaccine was evaluated in specific pathogen-free kittens (Veir 2006). This prospective, randomized, placebo-controlled study resulted in the recovery of *E. faecium* SF68 from the feces of 7/9 cats treated with the probiotic, and a non-significant increase in FHV-1-specific serum IgG levels. Concentrations of total IgG and IgA in serum were similar between the probiotic and placebo groups and the percentage of CD4 + lymphocytes was only significantly increased in kittens at 27 weeks and not at any other time points. Probiotics have also been evaluated in juvenile captive cheetahs, a population with a relatively high incidence of bacterial-associated enteritis. Administration of a species-specific probiotic containing *Lactobacillus* Group 2 and *Enterococcus faecium* to 27 juvenile cheetahs was associated with a significantly increased body weight in the treatment group, while there was no increase in the control group (Koeppel 2006). In addition, administration of the probiotic was associated with improved fecal quality in the probiotic group. It should be emphasized that all studies were performed in healthy kittens or cats, and there are no published studies to date evaluating the use of probiotics in cats with gastrointestinal disorders such as bacterial or parasitic-associated diarrhea, food allergy, antibiotic-associated diarrhea, or IBD.

### **Future considerations**

The potential benefits and specific indications for probiotics in dogs and cats have yet to be clearly defined, and our understanding of the nature and diversity of the canine and feline intestinal microflora during health and disease is slowly expanding. The diverse microbial content of the intestinal tract is not adequately reflected by fecal analysis which has been the predominant sample analyzed to date. The application of genome analysis to the study of the microbial ecology of the gastrointestinal tract should facilitate the identification of major culturable and non-culturable populations, and provide a tool for studying shifts in these populations over time and under different conditions. The completion of prospective, randomized, placebo-controlled studies in dogs and cats that rely on clinically relevant end points that relate to a particular physiologic or pathologic condition is needed to define a role for probiotics. Probiotics do now appear to have a potential role in the prevention and treatment of various gastrointestinal and allergic illnesses, but it is likely that benefits achieved are specific to the bacterial species used and to the underlying disease context. Further work will help us better define the appropriate probiotic species and the specific indications for their use.

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## NOTES

This image shows a single sheet of white paper with horizontal ruling lines. The lines are evenly spaced and run across the width of the page. There are no margins, text, or other markings on the paper.