Australian and New Zealand College of Veterinary Scientists

Dermatology Chapter
Science Week Proceedings:

Allergies, infections and everything in between
10th & 11th July 2015
THANK YOU

The Dermatology Chapter of the Australian and New Zealand College of Veterinary Scientists would like to thank Elanco for their generous sponsorship of Chapter scientific programs and social events in 2015.

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GOLD LEVEL SPONSOR
Science Week, Gold Coast 2015
12th Annual Dermatology Chapter Meeting of the Australian New Zealand College of Veterinary Scientists

This year we are privileged to have Stephen White from Davis, California presenting on a range of topics. Stephen would be one of the best-known veterinary dermatologists in the world, authoring over 90 scientific papers in his more than 30 years in dermatology. Stephen is joined by Nick Cave, from Massey University for a comprehensive presentation on adverse food reactions.

We also have some very talented speakers within the chapter and David Robson steps up again detailing a number of recent and ongoing studies. The worldwide issue of MRSP is continued this year. It will remain an ongoing and increasing challenge in the coming years and we need to keep up to date with its progress and treatment. Jacqui Norris and Darren Trott will give an update on the research presented last year. Finally we will have an overview of equine dermatology from Stephen White.

We hope you enjoy this varied and cutting edge program. We encourage you all to join us in Bordeaux next year for the World Congress in Veterinary Dermatology.

Karyn Wesselingh, Beth MacDonald
Debbie Simpson, Fiona Bateman, Meng Siak, Peter Hill, David Robson and Mandy Burrows
(Dermatology Chapter Science Week Coordinators)
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Biographies

Stephen White
Dr. White became a Diplomate of the American College of Veterinary Dermatology in 1983. A graduate of the School of Veterinary Medicine at the University of California at Davis, he also did his internship and dermatology residency there as well. He has held faculty positions at Tufts' School of Veterinary Medicine and Colorado State's College of Veterinary Medicine and Biomedical Sciences before joining the School of Veterinary Medicine at UC Davis as a full Professor in 1998. Dr. White has twice been a sabbatical professor at the National School of Veterinary Medicine in Nantes, France. His areas of interests include food allergy, cutaneous manifestations of systemic disease, rabbit/rodent dermatology, and equine dermatology. Dr. White has published over 90 scientific papers in refereed journals.

Nick Cave
Nick Cave graduated from Massey University (NZ) in 1990 with a BVSc, and worked in general practice for 6 years until 1997, when he returned to Massey for a residency in small animal internal medicine, and attained membership in the Australasian College of Veterinary Scientists by examination. He graduated with a Masters in Veterinary Science in 2000, entitled "The nutritional management of food hypersensitivity in dogs and cats: an assessment of a protein hydrolysate". In 2004 he moved to the University of California, Davis, where he attained a PhD in nutrition and immunology, with his thesis entitled "The role of estrogen in controlling food intake, and the efficacy and immunological effects of genistein as a surrogate estrogen, in cats following gonadectomy." At the same time, he completed a residency in small animal clinical nutrition, and became a diplomate in the American College of Veterinary Nutrition by examination in 2004. In late 2005, he returned to Massey University as senior lecturer in small animal medicine and nutrition. He has authored more than 30 peer-reviewed publications, is on the editorial board for the Veterinary Quarterly and Veterinary Education International, and has authored chapters in several international veterinary textbooks. He is a founding member of the WSAVA Nutritional Guidelines Committee.

John Hutt
John graduated from the University of Queensland in 1988. He worked in general practice in Brisbane for 18 months before travelling to the UK in 1990, where he worked in a small animal practice for the next 11 years. During this time he developed an interest in veterinary dermatology. He was awarded the RCVS Certificate in Veterinary Dermatology in 1997, and worked as clinical assistant in dermatology at the Animal Health Trust from 1998 to 2000. John is a past President of the British Veterinary Dermatology Study Group. In 2001 John set up North Kent Referrals, a multi-disciplinary referral practice in the south of England, and he owned and ran the practice until its sale in 2012. In 2006 John was admitted to membership of the Australian College of Veterinary Scientists (MANZCVS) by examination in small animal medicine. Following his return to Brisbane, John joined the team at Dermatology for Animals as Resident in Dermatology in July 2013. Outside of work John's major interest lies in English Literature and in 2006 he completed a Bachelor of Arts at the Open University.

Jacqueline Norris
Passionate about teaching clinically relevant infectious disease courses for veterinary undergraduates, veterinary practitioners and companion animal breeders that are centred on the relationship between host, pathogen and environment and how each is involved in the development of disease in animals (including humans). Her research reflects a broad interest in infectious and non-infectious diseases. This includes: Companion animal viral diseases (especially Feline Infectious Peritonitis, Feline Calicivirus, Feline immunodeficiency virus, Canine Parvovirus, Canine Distemper virus); Q fever (how common is it in the veterinary personnel and animal owners, what are the risk factors for exposure? What role do companion animals play?); Multi-resistant Staphylococcus species – what role do these Staphylococcus species play in companion animal disease (cats, dogs, horses), what are the preventable risk factors and the risks to health in human communities (pet owners, pet breeders, and veterinary personnel)?; and Chronic Renal Disease in Felids (big cats eg Lions; and domestic cats)- development of strategies for early diagnosis, targeted treatments and prevention.
David Robson
David graduated in 1992 from the University of Queensland. He worked as an associated at Chermside Veterinary Hospital, Brisbane, for six years and gained Membership of the Australian College of Veterinary Scientists in Medicine of Dogs in 1999. Later that year he took up residency at the Animal Skin Ear and Allergy service at the Melbourne Veterinary Referral Centre, Glen Waverley, under the supervision of Greg Burton, and passed his ACVS Fellowship examination in dermatology in 2005. His major publications include a two-part review on cyclosporine and its role in dermatology, the cytology of the normal anal sac and the longest case series to date of Demodex injai demodicosis. He has practiced clinical dermatology and lectured both in Australia and overseas.

Darren Trott
I completed my veterinary degree at Murdoch University and worked in small animal practice for eight years. Following an honours project on Listeria monocytogenes I became interested in the ecology of infectious diseases and completed a PhD with Prof David Hampson on intestinal spirochaetes (awarded in 1998). After completing a three year post-doc at the National Animal Disease Center in Ames, Iowa, USA, I accepted a lectureship at The University of Queensland (UQ) School of Veterinary Science in 2000. I taught veterinary microbiology to veterinary undergraduates at UQ for 10 years and conducted research on gastrointestinal diseases, microbial ecology and virulence/antimicrobial resistance in companion animal, livestock and human bacterial pathogens. I joined The University of Adelaide School of Animal and Veterinary Sciences in January 2010 and my research areas have expanded to also include the development of new antimicrobials for superbug infections.
Eosinophilic granuloma complex in cats and dogs

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The eosinophilic granuloma complex (EGC) in the cat actually consists of three similar diseases. Despite the name, the three diseases do not always cause granulomas. The presence of eosinophilic degranulation products have been documented coating but not altering the collagen in all of these diseases.

These diseases are best thought of as inflammatory reactions of the skin, often associated with hypersensitivity diseases. Thus, the veterinarian should attempt to search for any underlying diseases. [One article\(^1\) suggest that even *Felis domestica* allergen I (Feld I) could be an autoallergen responsible for chronic inflammatory reactions in cats with EGC.] Cat skin may respond with eosinophils to diverse a group of diseases as allergies, pemphigus, neoplasia, or pyoderma. Thus specific histological as well as clinical guidelines must be used to make the diagnosis of EGC.

The lip ulcer (eosinophilic ulcer, indolent ulcer, rodent ulcer) is found on the upper lip of cats. Diagnosis is based on clinical appearance as well as histopathology, which generally reveals hyperplastic ulcerative superficial perivascular dermatitis with eosinophils or neutrophils, mononuclear cells and fibrosis. Blood eosinophilia and tissue eosinophilia are less common than the other diseases in this complex. The major underlying diseases identified with the indolent lip ulcer are flea allergy\(^3\), food allergy and atopic dermatitis\(^3a, 3b\); when these are controlled, the lip lesion resolves. Occasionally infection with *Microsporum canis* may be responsible for lip ulcers.\(^4\)

The eosinophilic plaque is usually seen on the ventral abdomen or inner thigh. Typical lesions show raised erythematous orange to yellow plaques. Differential diagnosis must include both granulomatous diseases and neoplasia. Biopsy reveals hyperplastic, superficial and deep perivascular dermatitis with eosinophilia and at times a diffuse, eosinophilic dermatitis. Eosinophilic microvesicles and microabscesses may be seen in the epidermis. The eosinophilic plaque has been associated with the underlying diseases of food allergy, flea allergy and atopic dermatitis\(^3a\).

Feline eosinophilic granuloma occurs most commonly in the oral cavity or in a linear fashion on the back legs. A subset of this disease has been associated with mosquito bites\(^5\) and presents as nodules, with or without ulceration, on the face, ears and feet. This condition has also been seen on/within the chin of cats (feline chin edema; ‘pouty’ cat) and affecting the foot pads. Typically, the lesions have a papular to nodular configuration and histologically show granulomatous dermatitis with multifocal areas of collagen coated with the released substances from degranulated eosinophils (formerly known as ‘collagen degeneration’). Eosinophils are common in the biopsies from the face or oral cavity, and there may be a peripheral eosinophilia as well. Eosinophilic granuloma of the hind legs has been associated with the underlying disease of flea allergy; it has also been seen with an apparently genetic predilection in a colony of SPF (specific pathogen-free) cats, as well as atopic dermatitis\(^3a\). The author has seen cases affecting the foot pads which were associated with certain types of cat litter.

Definitive diagnosis of the eosinophilic granuloma complex MUST be made on histopathology. There are simply too many differential diagnoses, which may fool the clinician to make the diagnosis only on visual examination. These would include neoplasias (lymphoma, mast cell tumor, etc), proliferative, non-neoplastic conditions (plasma cell pododermatitis) and infections (herpes virus).

Important Differential Diagnoses

*Herpes virus dermatitis* is probably under-reported. Persistent ulcerative to necrotizing lesions develop on the face, most typically the nose\(^6\) Affected cats often have a history of stress, glucocorticoid administration, or chronic ocular or respiratory disease suggesting this skin disease is associated with reactivation of latent herpes virus infection. However, the respiratory signs may be subtle, and owners should be questioned carefully to determine this facet of the history.
Histopathology shows a marked vesicular and ulcerative eosinophilic infiltrate with intranuclear viral inclusion bodies in the epithelium. Unfortunately, the viral inclusions are not always readily seen, and the histology can bear some resemblance to idiopathic or mosquito bite-induced eosinophilic granulomas. Subsequent electron microscopy and polymerase chain reaction evaluation may confirm the virus to be feline herpes 1.

**Treatment:**
1) Subcutaneous alpha interferon (Intron A®: Schering; 1 vial contains 3 million units) has appeared beneficial in some cases, sometimes dramatically so. Dosage is approximately 1.5-2 million units/m\(^2\) (a 5 kg cat has a m\(^2\) body area of 0.29 meters, and thus would receive a dose of 290,000-580,000 units) given subcutaneously three times weekly for at least six weeks. Side effects are uncommon but malaise may be seen. May be difficult to obtain.
2) Concurrent lysine therapy could be considered: a colleague’s suggested dose is 250 mg BID for 30 days, then SID to maintain. Use non-propylene glycol containing tablets.
3) Recent anecdotal reports are promising for using topical 5% imiquimod cream (Aldara®, 3M Pharmaceuticals), a ‘local immunological response modifier’, in the treatment of feline cutaneous herpes infection. Cats generally only tolerate 2-3 treatments per week, due to irritation reactions. The drug comes as a dozen 0.25 gm packets. One packet may supply enough cream for two applications. Cost is around $120 per dozen. There is an anecdotal report of 2 cats developing reversible leucopenia following ingesting (via grooming) imiquimod cream, which was placed on their lesions.
4) Famcyclovir 40mg/kg tid thus a 5kg cat = ½ of a 500mg tablet tid. Effective – now available as a generic.

**Plasmacytic pododermatitis** is a rare idiopathic cause of foot pad swelling and ulceration in cats. The initial condition is a soft, nonpainful swelling of the footpads, which may progress to ulceration and granulation tissue. Ulceration may lead to pain and lameness. Usually the metacarpal or metatarsal pads are involved but the digital pads may also be affected. Diagnosis is by biopsy, which reveals a diffuse dermatitis with a massive plasma cell infiltration. This disease may be associated with FIV infection. The best mode of therapy is still undetermined. Corticosteroids have not always been beneficial. Some cases will regress spontaneously. Doxycycline, 5 mg/kg q 12 h, gold therapy in a regimen as for plasmacytic-lymphocytic stomatitis or surgical removal of affected tissues, are treatments that have been reported as effective. Reports from Europe indicate a high percentage (50%) of these cats are FIV-positive. Researchers using both immunohistology and PCR were unable to identify any organisms in the lesions.

**Treatment of feline EGC**
Traditionally, these diseases have been treated with intra-muscular injections of methylprednisolone acetate (Depomedrol®:UpjohnPharmacia) at 4 mg/kg, given once every two weeks for three injections. The author uses this treatment ONLY IF: the disease has been confirmed by biopsy, there is no evidence of, or no ability to investigate, an underlying cause (especially feline herpes virus dermatitis), and this protocol is only used twice a year at most.

More frequent use of this protocol will lead to the development of diabetes in a very high percentage of cats. If further corticosteroid treatment is needed, oral prednisolone, initially at a dosage of 1 mg/kg q12 h may be used, then tapered to the lowest effective dosage.

In an attempt to avoid corticosteroids, the following treatments have been reported/utilized:

In one study, 4 of 4 eosinophilic granulomas, but 0 of 2 eosinophilic plaques were shown to respond to administration of essential fatty acids (DermCaps®:DVM Pharmaceuticals (now Bayer)). Dosages approximated the manufacturer's guidelines. These are well-tolerated medications.

Cyclosporine: a good response to a dose of 25 mg/cat was seen in 6 cases of eosinophilic plaque and 3 cases of oral eosinophilic granuloma in one report. In three cases of indolent lip ulcers, the response was less impressive. Another more recent study confirmed these results with a higher dosage range of 10-12.5 mg/kg.

If dermatophytes are present, fluconazole or itraconazole (10 mg/kg q24 h) should be used – previous anecdotal reports of lip ulcers’ responses to griseofulvin may in fact have been due to an underlying *M. canis* infection.

Clavamox has been reported as effective in the treatment of eosinophilic plaques.
Canine Eosinophilic Granuloma Complex

**Canine eosinophilic granuloma** is most commonly reported in Arctic Circle breeds and Cavalier King Charles Spaniels, and is most often seen on the inner thighs or in the mouth. Erythematous to yellow raised nodules with papillated surfaces are typical. Pruritus is variable. Diagnosis is by histology, which is identical to the feline eosinophilic granuloma, with eosinophils and granulomatous inflammation around eosinophilic debris-coated collagen. Treatment is with prednisone at 1 mg/kg q12h for one week, then tapering down over the course of four to six weeks. Occasionally, higher initial dosages are necessary.

**Canine eosinophilic furunculosis** is a closely-related disease. It has been reported in many breeds, but typically is seen in long-nosed large breeds or curious small breeds (i.e., terriers) with potential access to wasps, bees, ants, spiders, etc. It is thus felt to be due to arthropod bites or stings. Consistent with this, the disease may be very rapid in onset, leading to nasal/muzzle swelling, exudation and pain. Large, swollen, erythematous lesions on the muzzle are the most common lesions, but in some dogs similar lesions may be seen on the head, pericarially and around the pinna. Impression smears will often show eosinophils. While diagnosis is usually done on a clinical basis, histologic confirmation will show lesions similar to that of the canine eosinophilic granuloma, but with more eosinophilic infiltration into the epidermis and follicular wall, a furunculosis, and fewer areas of eosinophilic debris-coated collagen. Treatment is as reported for the canine eosinophilic granuloma.

**References**


The immunology of cutaneous food hypersensitivity
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The most widely celebrated early demonstrations of the mechanism of food hypersensitivity was by a German physician, Carl Prausnitz, who theorised that there was a "transferable factor" in the blood of his graduate student, Heinz Kustner, who was allergic to fish. To prove this, he injected serum from Kustner under the skin of his forearm, and then showed that "pricking" the skin with a tiny amount of soluble fish protein produced a wheal at the site. Eating fish temporarily also produced a wheal, thus illustrating that intact food proteins can circulate systemically following ingestion.

Most hypersensitivity diseases manifest clinical signs in the same region that it is supposed sensitization occurs. Allergic rhinitis, allergic conjunctivitis, contact hypersensitivity sensitivity, and flea-bite hypersensitivity, are good examples of the region-specific induction and manifestation. Patients with allergic gastroenteritis are another example. Systemic anaphylaxis following allergen ingestion can be explained by the widespread distribution of allergen-specific IgE, which leads to sensitisation of mast cells and other cells bearing the high-affinity IgE receptor (FcεRI), and hence widespread or systemic responses when the allergen circulates in the blood. However, many cases of food hypersensitivity manifest clinical signs in tissues remote from the gastrointestinal tract. Cutaneous signs have been suggested to accompany gastrointestinal signs in up to 65% of cases of food hypersensitivity, and although there is no accuracy to such estimates, there is no doubt that such presentations exist. Moreover, food hypersensitivity frequently manifests clinical signs in the skin, in the absence of any signs of hypersensitivity in the gastrointestinal tract (cutaneous food hypersensitivity, CFH). In a recent study of cats with CFH, only 2.1% had concurrent gastrointestinal signs. In fact, some authors state that cutaneous signs are more common than GI signs, though that claim is not supported by any published study, and the nature of bias presentations and reporting makes it very difficult to even speculate. None-the-less, CFH exists, and the explanation of its immunopathogenesis remains enigmatic.

The prevalence of food allergy in people appears to be increasing in the developed world. There is no evidence that recommendations to avoid ingestion of common food allergens in early life have helped reduce the prevalence, and some have suggested that the practice may have in fact contributed to the increase. This suggestion is based on the concept that early oral exposure leads to oral tolerance, whilst oral avoidance leads to lack of oral tolerance and the potential for sensitization via different routes.

Immunological basis for oral tolerance
Foreign dietary antigens interact with the intestinal immune system in such a way as to prevent unnecessary and detrimental immune reactions to them. In so doing, systemic immunity is rendered effectively unresponsive if the same antigen reaches the systemic circulation. This absence of reactivity to orally administered antigens is termed oral tolerance. Oral tolerance is generated in an antigen-specific and active manner that involves the induction of an atypical immune response. In the normal intestine, dendritic cells (DCs) lack co-stimulatory molecules, and when they present antigens to naïve B and T cells within the follicle, it occurs within a local microenvironment that differs from other sites in the body. The result is the induction of hyporesponsive, regulatory (Treg) or Th2 biased T cells. Activated cells then leave via lymphatics and pass via the mesenteric lymph nodes into the systemic circulation. They will then exit at mucosal sites via engagement of cellular adhesion molecules (CAMs) specifically expressed by the high-endothelial venules of mucosal tissues. Thus activated or memory B and T lymphocytes enter the lamina propria to await a secondary encounter with their specific antigen.

The activated cells may secrete cytokines, but full differentiation into effector T cells or plasma cells may not occur without secondary exposure. For both cell types to be re-exposed to antigen, intact antigens must reach the lamina propria. Intestinal epithelial cells are responsible for the absorption of antigen, release to professional APCs, and limited antigen presentation to cells within the mucosa on MHC class II. In the normal intestine, these secondary APCs will, like the primary presenters, lack co-stimulatory molecule expression and further add to the tolerogenic environment. The effector T cell clones resident in the normal intestine secrete a bias towards Th2 and Treg cytokines, in particular IL-10 and TGF-β, thus directing B-cell isotype switching to produce IgA-secreting plasma cells, whilst inhibiting the development of Th1 lymphocytes and IgG production.
This combination of apoptosis, functional defects in surviving clones, and T cells secreting the anti-inflammatory and IgA-supporting cytokines, is the general basis for immunological tolerance to luminal antigens. Thus oral tolerance is composed of a delicate balance between induction of IgA, T cell deletion, anergy, and immunosuppression; and the retention of antigen-specific lymphocytes capable of responding to invasive pathogens though antibody isotype switching to IgM, IgE, or IgG, and the production of inflammatory cytokines such as IFN-γ, IL-12, and IL-6.

**Loss of oral tolerance**

The initiating events that lead to loss of oral tolerance, or prevent it from developing have not been defined in dogs or cats, and remain poorly understood in any species. Suggested mechanisms include:

- **A)** Increased mucosal permeability e.g. following mucosal injury, or the neonatal intestine,
- **B)** Co-administration of a mucosal adjuvant that activates and changes the phenotype of intestinal dendritic cells e.g. bacterial enterotoxins
- **C)** Parasitism: Intestinal parasitism in cats leads to an exaggerated systemic humoral response that includes increased production of IgE.⁵
- **D)** Nutritional modification: In people, there is an association between vitamin D status and allergic sensitisation.⁶ No association has been tested for in dogs or cats.

None-the-less, it is widely assumed that the aberrant immunological event occurs within the intestinal mucosa. What is lacking in that assumption, is the ability to explain how a loss of mucosal tolerance to an ingested antigen can result in CFH, rather than clinical signs of gastrointestinal disease. What explains those cases where only the skin is affected?

Could cutaneous signs be due to aberrant lymphocyte migration? Lymphocytes activated within the intestinal mucosa exit via lymphatics and pass via the mesenteric lymph nodes into the systemic circulation. A unique feature of these cells is the expression of the integrin α4β7, which specifically engages with the cellular adhesion molecule MAdCAM-1, specifically expressed by the high-endothelial venules of mucosal tissues.⁷ Thus B and T lymphocytes activated within the mucosa reenter the lamina propria and other mucosal sites to await a secondary encounter with their specific antigen. Lymphocytes activated in peripheral sites are usually induced to express the integrin α4β7, and will therefore not enter mucosal sites producing an effective compartmentalization of the specific immune system. Consistent with this is the finding that human patients with IgE-mediated milk hypersensitivity leading to gastrointestinal signs, have increased expression of α4β7 in circulating β-lactoglobulin-specific T cells.⁸

It might be that in CFH, allergen-specific T cells inappropriately circulate to non-mucosal tissues where their natural Th2-bias induces the local production of IgE.⁹ The cutaneous analogue to α4β7 is the cutaneous lymphocyte antigen (CLA), which binds to E-selectin. Expression of E-selectin is absent in normal canine skin, but is up-regulated in response to TNF-α, and CLA is highly expressed on T cells infiltrating skin in a variety of causes of dermatitis.¹⁰,¹¹ In children with milk-induced urticaria or eczema, circulating lymphocytes up-regulate CLA after in vitro stimulation with casein, and have significantly more CLA-positive circulating cells than patients with intestinal food hypersensitivity.¹²,¹³ Thus during hypersensitisation, there appears to be an expansion of CLA⁺ allergen-specific T cells accompanied by an up-regulation of its ligand, E-selectin, in response to local TNF-α and histamine production. Interestingly, vitamin D (and retinoids) inhibit CLA expression, and low vitamin D and/or A status may increase the risk of aberrant migration to the skin.¹⁴ However, aberrant migration would still require aberrant activation of food-specific lymphocytes in the intestine, thus this cannot be a primary cause.

**Could local IgE production be the cause of CFH?** In many, though not all patients with CFH, allergen-specific IgE is present in circulation, which seems incongruent with cases that present with only cutaneous signs. Mast cell density is similar in the uterus and lung and throughout much of the intestinal tract of dogs when compared to that of the skin.¹⁵ Therefore, the systemic production of IgE should, theoretically, result in multiple organs being affected or even systemic signs, as seen in certain models of induced hypersensitivity.¹⁶ That this does not occur is consistent with the poor correlation between circulating food allergen-specific IgE and clinical reactivity to challenge with those allergens.¹⁷ The limitation of clinical signs to cutaneous signs could be explained, however, if the majority of the allergen-specific IgE is produced by plasma cells within the dermis, or if IgE is an epiphenomenon and not a significant contributor to clinical reactivity.

**The skin as the primary induction site of cutaneous food hypersensitivity** It has previously been assumed that sensitisation to food allergens occurs through the intestinal tract. However, the difficulty in explaining CFH in light of conventional understanding of oral tolerance and lymphocyte migration suggests we should question that assumption. Antigens absorbed through the dermis are ingested by Langerhan’s cells, which migrate to regional lymph nodes and present antigen to T cells there. Activation of T cells in those nodes induces the expression of CLA and sequestration
of antigen-specific T cells into regions of the dermis where local inflammation has resulted in the up-regulation of E-selectin.

Sensitisation through the dermis is easily demonstrated, and can lead to local, and even systemic IgE production resulting in allergen-primed mast cells in the dermis and other tissues. Oral feeding of the allergen can then produce cutaneous signs, or even systemic anaphylaxis. A pre-requisite for this to occur is the lack of oral exposure prior to epicutaneous sensitisation, whereby the induction of oral tolerance lessens, or may even prevent dermal sensitisation. Likewise, small doses of food allergen applied to a disrupted or abnormally permeable dermis can prevent the development of normal oral tolerance when that food is subsequently ingested in mice. Finally, the combination of barrier disruption and the administration of a bacterial adjuvant with the protein produces the most robust and consistent systemic and local sensitisation and IgE induction. Increased permeability to proteins is well established in atopic humans, and has been established in atopic dogs. Perturbation of the barrier function of the stratum corneum may stimulate inflammation, epidermal hyperplasia, entry of allergens, and serve as a natural sensitization pathway for food allergy.

Increased epidermal permeability is clearly associated with atopic dermatitis, and associations between mutations in filaggrin genes and an atopic predisposition supports that it is causal. To further support the hypothesis that cutaneous sensitisation is operative in CFH, several recent studies (though not all) have shown associations between variants in the filaggrin gene and food allergy in people. In a recent study, almost 90% of children with specific filaggrin gene variants had clinically proven food allergy. Indeed CFH and canine atopic dermatitis (CAD) are elicited following ingestion. It may also explain the close clinical association between atopic dermatitis and CFH. Increased permeability to proteins is well established in atopic humans, and has been established in atopic dogs. Perturbation of the barrier function of the stratum corneum may stimulate inflammation, epidermal hyperplasia, entry of allergens, and serve as a natural sensitization pathway for food allergy.

Once hypersensitivity is established, IgE in the systemic circulation, has the opportunity to bind to any cell expressing FceRI, not just mast cells. Other cells include, importantly, the Langerhans cells. The loading of IgE on APCs greatly increases their efficiency of presentation of circulating allergens, by increasing the internalisation and processing of the specific allergens. When activated in such a manner, Langerhans cells induce a Th2 bias in the T cells they present to. In this way, CFH might develop if systemic IgE is produced in response to abnormal mucosal responses to dietary antigens, but subsequent antigen presentation in the GIT might resolve local responses. However, the period of IgE production might sensitise Langerhans cells, and if accompanied by cutaneous allergen absorption might lead to a secondary antigen presentation to locally resident reactive T cells if they exist.

These concepts support the idea that CFH is a cutaneous allergy, and not an "oral allergy, even though clinical signs are elicited following ingestion. It may also explain the close clinical association between atopic dermatitis and CFH. Indeed CFH and canine atopic dermatitis (CAD) can be clinically indistinguishable, and both may be present in the same dog. In dogs experimentally sensitized to house dust mites demonstrated exacerbations of their atopic dermatitis when they were challenged orally with storage mites, which are known to cross react immunologically with dust mites. In those dogs, the distribution of pruritis (face, pinnae, feet and ventral abdomen) was indistinguishable from that elicited by epicutaneous challenge with dust mite allergens. In a review of 266 atopic dogs diagnosed using IDST, it was found that 7% had a concurrent adverse reaction to food. In a study of dogs diagnosed as atopic based on the Willemse definition, 19/62 (31%) of the dogs that were subjected to an elimination-challenge diet trial were found to have an adverse food reaction.

Is then, CFH simply atopic dermatitis but the allergens are food proteins? The relationship between CFH and atopic dermatitis is still difficult to define. For some animals, ARF can exacerbate AD and, consequently, some cases of CFH manifest clinically as CAD. Both diseases appear to have the same route of sensitisation, both can be exacerbated by oral ingestion with the same cutaneous distribution, and barrier disruption increases risk of sensitisation to both food and "environmental" allergens.

Some ex vivo immunological tests support the hypothesis that CFH and CAD share an immunopathogenesis. Activation of circulating lymphocytes following in vitro stimulation with food allergens has been found to be strongly associated with food hypersensitivity, but reactions in dogs with CAD is also common. Interestingly, following an elimination diet, although there was no change in food-specific IgE, the allergen-specific lymphocyte responses were absent. In another study, activation of circulating lymphocytes was demonstrated in 91% of a group of dogs with clinically-defined atopic dermatitis which were without food-specific IgE. However, in a recent study of the histology of dogs with CFH, the dominant lymphocyte type in lesional skin was a CD8+ T cell. Thus, the effector mechanisms that operate in pure CFH may be different to AD sensu stricto.

Many questions remain. Are the effector mechanisms in CFH IgE-dependant? If so, is that the case for all, or just a proportion? Although some patients with CFH will react to IDST and some have elevated food-specific IgE, many do
not. It is impossible to conclude from such examples whether that is evidence against IgE-mediated disease, or if it is due to inappropriate allergen selection or test methodology. The lack of standardisation between laboratories and poor concordance in results highlights the difficulties with allergen-specific testing. These questions clearly require answering if laboratory testing is to be useful for the diagnosis of CFH. Statements about sensitivity and specificity too often don’t take into account the possibility of inadequate antigen preparation, or incorrect selection.

It is almost 100 years since the initial report of Prausnitz, demonstrating that a soluble factor in serum (IgE) from a food allergic patient could be transferred to the dermis of another patient inducing sensitisation at the site of injection. Immediately after ingestion of the appropriate meal, a local wheal response developed. Not only did this demonstrate the functional nature of IgE, but also that systemic absorption of allergens occurs soon after a meal, and can react with IgE-laden mast cells in the dermis. If CFH is due to cutaneous sensitisation, it opens the intriguing possibility for strategies that employ the induction of oral tolerance, such as gradual reintroduction of the offending allergen, might be successful therapeutic approaches.

References


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Long term management of food hypersensitivity
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When food hypersensitivity is diagnosed by elimination and challenge diet trials, it is rare, even in academic institutions that any attempt is made to demonstrate an immunological basis for the clinical reactivity. Indeed in many cases, owners may be understandably reluctant to conduct challenge trials, and all that is known is that there was improvement coincident with dietary change. A proportion of cases that do not have food hypersensitivity may improve with dietary change, and those cases may be misdiagnosed as hypersensitive if not challenged. Reasons for improvement in those cases includes the effects of reintroducing previously deficient essential nutrients, improvement in barrier function, and the immune modulatory effects of nutrients such as polyunsaturated fatty acids and vitamins D and A. It is not known how often hypersensitivity is misdiagnosed, but in the absence of challenge trials and assays that demonstrate an immunological basis to the reactivity it is likely that it occurs.

For that reason, it is usually more precise to diagnose cutaneous adverse reaction to food (CARF), rather than cutaneous food hypersensitivity (CFH). Thus, the long term management of patients with suspected food hypersensitivity will be for patients that range from those that have simply improved on an elimination diet, to those in which the specific allergen has been defined, and an immunological basis has been demonstrated. Despite this range of diagnostic certainty, the two primary concerns for the long term management of food hypersensitivity are: avoidance of the known or suspected allergen(s), and meeting the nutritional requirements of the patient. Thus, the long term management requires that an appropriate diet is selected, and a decision made about how long that diet should be complied with.

Nutritional considerations
Long term feeding of a specific diet should only be recommended if the diet is known to be complete and balanced, which can be demonstrated by evaluation of published analyses of the ingredients, chemical analysis of the diet, or through a feeding trial. Diets may be defined as being "Formulated to meet the requirements of" the intended animal, or defined as having passed a suitable feeding trial. Both the American Association of Feed Control Officials (AAFCO) and the European Pet Food Industry Federation (FEDIAF) have well established protocols for conducting feeding trials, and diets that have been subjected to these can be held to be truly complete and balanced. It is the author's opinion that veterinarians should recommend diets that have passed one of these feeding trials if a suitable diet exists. An acceptable alternative is a diet that is formulated to meet the requirements of the animal, as established by AAFCO or FEDIAF. If the pertinent clinical signs have been acceptably controlled on a AAFCO/FEDIAF tested diet, then the simplest approach is to maintain that elimination diet in the long term, whilst considering the points below.

Home prepared diets
Home prepared diets (HPDs) are frequently used as elimination diets, usually consisting of a single novel protein. Since most veterinarians prescribe these HPDs for up to 10 weeks for the diagnosis of CFH, it is worth considering the significance of nutritional deficiencies encountered during that feeding period in an adult dog or cat (see table below).

Key nutrient deficiencies and excesses in a diet of venison meat only for a cat.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percentage of NRC requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>3%</td>
</tr>
<tr>
<td>Manganese</td>
<td>20%</td>
</tr>
<tr>
<td>Iodine</td>
<td>0%</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0%</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>25%</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>0%</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>230%</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>1,891%</td>
</tr>
</tbody>
</table>

*Nutrient requirements of dogs and cats, National Research Council."
That dogs and cats do not apparently become systemically ill with short term feeding of such diets is credit to several factors including:
- Our inability to measure the effects of short-term nutritional inadequacy
- The uncertainty of the absolute physiology requirements
- The difference between long term requirements averaged out as a daily requirement, vs. a true short-term requirement
- Non-compliance, and the pragmatic tendency for a varied diet to be more likely to be complete than a restricted one

Although not properly surveyed, most incomplete or unbalanced diets used in veterinary dermatology are based on a primary meat source, chosen as a novel protein, such as horse, rabbit, lamb, or duck meat. These diets are characterised as being higher protein, variable fat, and little to now carbohydrate compared with conventional commercial diets. Such diets will be very high in protein, B-vitamins, phosphorus, but grossly deficient in calcium. Thus the most commonly reported clinical effects from meat-based diets are due to osteopenia and fibrous osteodystrophy from inadequate calcium.

In Europe, a survey of HPDs fed to dogs found that energy, fat and protein were above AAFCO recommendations, whereas calcium, Ca:P ratio and vitamins A and E, and potassium, copper and zinc concentrations were below recommendations. Relative fatty acid contents of serum phospholipid fractions of HPD-fed dogs were significantly lower in 18:2(n-6) and 20:4(n-6) than those from a population of 37 normal dogs consuming commercial dry, US-manufactured diets, although one might argue, especially for chronic dermatitis, that might be a good thing.

In the USA, the great majority (>90%) of HPD recipes used by veterinarians and owners are nutritionally inadequate. Unfortunately, even textbook and refereed publications may not contain adequate recipes. In one study of 49 maintenance and 36 growth diets published in 6 veterinary nutrition books, the majority had one or more significant nutrient deficiency. The most common nutritional inadequacy was a deficiency of calcium and too low Ca:P ratio, which is an inevitable consequence of an unsupplemented meat-based diet. Other common deficiencies included vitamins B12, E, D, and A; copper, manganese, and iodine. Common excesses included total fat, 18:2(n-6), and B-vitamins.

Malnutrition is more likely to occur during growth than in an adult, and it is important to realise how quickly significant clinical disease can develop. It may be many months before a severely calcium deficient diet causes clinical signs of osteopenia in an adult. In contrast, severe signs of fibrous osteodystrophy can develop within 6-8 weeks in kittens and puppies when fed deficient diets during the first 3-6 months of life. Not all commercial diets marketed for the diagnosis of food hypersensitivity are suitable for long term feeding to growing animals.

HPDs can, and should, be formulated to be the equal of commercial diets. However, a combination of inadequate recipes, owner (and veterinarian) ignorance, and the natural tendency for “recipe drift” combine to produce a large proportion of inadequate diets. Proper formulation of HPD recipes can be performed by consulting a veterinary nutritionist through either the American College of Veterinary Nutrition, or the European College of Veterinary Comparative Nutrition. Alternatively, recipes can be created using a web-based service created by a veterinary nutrition specialist (www.balanceit.com). Most properly formulated HPDs contain a minimum of 6, and up to 9 separate ingredients. Although not studied, client compliance probably decreases with increased ingredient list. Formulating complete diets without using “supplements” is perfectly possible, but requires many more ingredients.

**Feeding the largest organ**

Almost any essential nutrient would cause or exacerbate cutaneous disease if deficient. However, most essential nutrients will cause serious clinical signs in other organs long before the skin is affected. Thus, we are interested in nutritional deficiencies that are 1) common, 2) serious, and 3) may manifest with cutaneous signs prior to other organs. Nutritional deficiencies can be grouped according to those that are highly likely to occur, those that can occur but are rarely seen, those that are likely, but not currently recognised, and those that we frequently recognise. The table below groups some of the essential nutrients that can be deficient with meat-based diets according to their likelihood.
Table 2. Clinically apparent nutritional deficiencies possible on "meat-based" elimination diets grouped according to likelihood.

<table>
<thead>
<tr>
<th>Highly unlikely</th>
<th>Rarely seen</th>
<th>Likely but unrecognised</th>
<th>Common and important and</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium, essential amino acids, folate, vitamin K, riboflavin</td>
<td>Essential fatty acids (linoleic acid, arachidonic acid), thiamine, vitamin E, selenium, taurine</td>
<td>Vitamin A, vitamin D, iodine, copper, manganese</td>
<td>Calcium, vitamin D?,</td>
</tr>
</tbody>
</table>

Essential fatty acids.

It is now well recognised that atopic dermatitis is associated with impaired ceramide production within the stratum corneum, which leads to increased water loss, and likely increased allergen absorption.\(^9,10\) The effect of the diet on ceramide production has not been carefully studied in dogs or cats. However, in one study, enrichment of the diet with a mixture of fatty acids (including LA, GLA, EPA and DHA) resulted in an increase in both free, and protein bound ceramides, cholesterol, and free fatty acids in and on the dermis.\(^11\) In another study, a commercial dry food based on potato, fish, and animal fat (Eukanuba Response FP), was compared with a home prepared diet of fish (cod or hake) and potato.\(^12\) Disease severity scores improved within 4 weeks of being fed the commercial diet. Although the study design precluded conclusions as to the mechanism of improvement, the commercial diet contained more LA, EPA, and DHA than the home prepared diet.

In addition to alterations in ceramide production in the dermis, atopic dogs appear to have altered fatty acid metabolism presumably in the liver. Atopic dogs appear to have reduced fatty acid desaturase activity, suggesting an impaired ability to produce long chain desaturated fatty acids.\(^13\) This could indicate that extra benefits may be seen from feeding higher concentrations of longer chain polyunsaturated fatty acids (20 to 24 carbon PUFA) than is required by normal animals. However, it is not known if these findings also apply to patients with CFH.

Many, perhaps most studies of the efficacy of PUFA supplementation on allergic dermatitis are hampered by failure to consider the dietary fat content concurrently ingested by the trial subjects. In one of the few to evaluate supplementation of a controlled diet, an n-3 PUFA supplement enabled a significant reduction in the use of prednisone required to control pruritis after 8 weeks of supplementation.\(^14\) In another study of atopic dermatitis, supplementation with flax seed or fish oil resulted in clinical improvement without changing the total FA content in skin biopsies.\(^15\) It may be that whole tissue change doesn't occur in 10 weeks, although the authors showed that the plasma concentration does, and perhaps superficial lipids of the stratum corneum do too. The Task Force on canine atopic dermatitis stated in regards to EFA supplementation that "As their mode of action requires their incorporation into cell membranes, a phenomenon that necessitates several weeks of treatment, essential fatty acids (EFA) are unlikely to be of any benefit for acute flares of AD in dogs".\(^16\) It is argued that it is far too early, and the waters of experimentation far too muddied for such a confident claim of either an exclusive effect of membrane incorporation, or a lack of rapid efficacy.

**Dietary influence on Th-2 development**

Several nutrients influence lymphocyte development and the commitment towards a specific T-helper immunophenotype. There may be specific concentrations of these nutrients that will assist resolution of clinical signs and the development of oral tolerance to dietary and other allergens. Vitamins D, A and E can influence T-lymphocyte development and cytokine production, and epidemiological studies in humans have implicated both low and high dietary concentrations of vitamin D as a risk factor for allergic disease.\(^17,18\) In addition, vitamins A and D can affect lymphocyte homing to the dermis, and dietary vitamin D can selectively decrease the expression of cutaneous lymphocyte antigen (CLA) without affecting homing to other tissues.\(^19\) At present, it is unknown which, if any nutrients are especially important in canine and feline food hypersensitivity and thus it is not possible to recommend one dietary approach over another. However, until the effective concentrations have been established, it is prudent to avoid deficiency or over-supplementation beyond normal dietary concentrations.

**Eventual restoration of tolerance to allergens**

Hypersensitivity to dietary antigens is not necessarily a life-long state. Although not clear, the expert opinion is that in humans, food allergies that start in childhood are often outgrown, whereas food allergy that starts in adulthood often persists.\(^20\) In people with IgE-mediated hypersensitivity to cow's milk, it is predominantly a disease of early childhood, which resolves within a few years. In a recent study of cow's milk allergy in children, almost 60% were no longer clinically allergic by 5 years of age, and the majority of those patients were no longer allergic within the first 2 years.\(^21\) In another larger study, the rate of resolution was less, with 19% resolved by age 4 years, 42% by age 8 years, 64% by
In a study of soy allergy in children, 25% had resolved by age 4 years, 45% by age 6 years, and 69% by age 10 years. In contrast, only about 21% of children that acquire peanut hypersensitivity will become tolerant by age 20, and only 9% of a study of 278 children with tree nut allergy were tolerant at adulthood.

In one study of food allergic dogs, there was no significant decrease in circulating food-specific antibody concentrations during a 10 week diet elimination trial in dogs. Similarly in another study, there was no change in food-specific IgE concentrations after 6-20 weeks of an elimination diet. However, activation of circulating lymphocytes following in vitro stimulation with the incriminating food allergens was absent at the end of the elimination diet period.

The resolution of confirmed food hypersensitivity in dogs and cats has not been well described. In study of 55 cats with chronic vomiting and/or diarrhoea, 16 cats were diagnosed as having food sensitivity based on elimination-challenge trials. However, a further 14 cats responded completely to an elimination diet, but did not recrudesce during a challenge with the staple diet. Whilst some of those cats may not have been challenged with the offending food allergen due to an incomplete dietary history, some cats may have rapidly re-established oral tolerance. It is possible that following a period of intestinal quiescence, those cats became clinically tolerant to the food protein, despite potentially still having sensitised antigen-specific lymphocytes.

A similar study in feline or canine food allergic patients with CFH has not been published. Indeed, although the author has seen several cases in clinical practice where there has been an apparent resolution, there are no published cases of food hypersensitivity that were shown to have subsequently resolved. The important questions that remain to be answered include how often does food hypersensitivity naturally resolve, how long does an elimination diet need to be fed before resolution could be expected and what are the risk factors for resolution?

**Hidden antigen sources**

The same difficulties in conducting an elimination diet trial are present in successfully feeding a diet for long term maintenance. There is great variation between manufacturers and products in how accurate the ingredient list is. Ingredient lists on many diets can be incomplete, or misleading. For instance, it is common for plant-derived ingredients to be included in diets in which only animal by-products are listed. Ambiguity or inaccuracy of ingredient lists, proteins in non-protein ingredients, the feeding of treats and chews, and scavenging present constant challenges. The difficulty obtaining owner compliance with weight management plans is testament to how often diet recommendations are not kept to.

**Recipe drift**

An unpublished phone-survey of clients of the Nutrition Consulting Service of the University of California, Davis, found that within weeks of starting a prescribed HPD, the great majority of clients modify, add commercial food, or abandon the diet completely. Clinicians who prescribe HPDs should be aware of this long term limitation, and if relapse of clinical signs occurs, careful re-evaluation of what is actually being fed is warranted.

**Supplementation of a diet with PUFA**

Dietary enrichment with n-3 PUFA can have immediate effects on immunity (e.g. antagonism of LPS signalling), but will take several weeks before a maximal response is achieved (i.e. saturation of tissue cell membranes). And although the effects and mechanisms of modulation of immunity by dietary lipid are complex, there is value in the generalization that diets enriched in n-3 PUFA reduce inflammation relative to diets enriched in n-6 PUFA. However, the effect a given diet will have is dependent on many dietary and animal factors, and the reduction of the description of the fat content of a diet to a simple ratio of n-6 to n-3 PUFA provides very limited and potentially misleading information.

Supplementation of a diet with a source of n-3 PUFA will have greatly varying effects depending on the nature of the basal diet and patient. Most commercial diets are highly concentrated in n-6 PUFA, and the addition of a small amount of n-3 PUFA (e.g. as marine fish oil), such as is contained in many veterinary fatty acid supplements, achieves little. The best approach is to start by feeding a diet that is already enriched in EPA, and not excessive in ARA. Several commercial diets are already enriched in n-3 PUFA, including Hill’s j/d, Nestle-Purina JM, Royal Canin Mobility, Royal Canin Skin Support, and Eukanuba Dermatosis FP. None of the currently available diets will produce a maximal immunosuppressive effect, and fish oil can be added to the enriched diet.

A recommended total fish oil dose is 0.2% to 2% of diet by weight per day, or a maximum of 0.4g EPA/100 kcal, including the n-3 content of the diet. Note that the ratio of EPA to DHA in fish oil varies between 1:1 and 3.5:1.
Conclusions and recommendations

It is frequently difficult to find diets that are truly novel to a patient. For long term feeding after a diagnosis has been made, only diets that are complete and balanced that are appropriate for that life-stage should be recommended. Although growing animals are more susceptible to malnutrition, clinical disease is still seen in adult animals on deficient diets. Clinically significant signs of nutritional deficiency can occur within a few weeks on some commonly used elimination diets. Home-prepared recipes offer flexibility and allow for the formulation of a truly novel diet, however they need to be properly formulated, and owners frequently alter recipes in the long term. Multiple nutrients have the potential to affect short and long term allergic disease but we do not know enough yet to make recommendations about supplementation or restriction beyond the normal ranges used by respectable manufacturers. Until more is known, the long term management of food hypersensitivity should focus on a few basic tenets:

- Feed a proven commercial complete and balanced diet suitable for the life-stage if possible, to avoid excess or deficiency
- If using home-prepared recipe, ensure it is properly balanced, and be aware of poor long term compliance
- Resolution of food hypersensitivity is possible, but it is not known how commonly or when it might occur
- If there is recrudescence of clinical signs on a maintenance diet, consider lack of compliance, hidden antigens, or sensitisation to the new diet

References


17. Litonjua AA. Vitamin D deficiency as a risk factor for childhood allergic disease and asthma. Current Opinion in Allergy and Clinical Immunology 2012; 12:179-185.


I. ‘Sam’ 10 year old neutered male DSH
History: 6 months of progressive pruritic exfoliative dermatitis. No other pets in household. Owners unaffected.
Previous diagnostics: Wood’s lamp (negative). Biopsy for histopathology: chronic active mononuclear perivascular dermatitis
Differential diagnoses:
Cheyletiella infestation
Dermatophytosis
Pemphigus foliaceus
Exfoliative dermatitis associated with thymoma
Cutaneous lymphoma
Others??
Diagnostics performed: skin scraping (negative), dermatophyte culture (negative), presumptive treatment with ivermectin for Cheyletiella infestation (0.3mg/kg per os q 2 weeks x 3): no effect.
Biopsy (total of 12 punch biopsies over a 6 week period: chronic active perivascular dermatitis
Responded to lamb baby food.

Take home messages:
1. Even if an old animal looks bad, strive for the diagnosis
2. If a case looks like ‘the worst case of XYZ that I have ever seen’ -- it’s probably not XYZ.

II. Lovey’ 4 year old spayed Labrador Retriever-cross
History: 4 months of raised, ulcerated plaques ventral to vulva. Dog is licking the area, but not constantly.
Previous diagnostics: biopsy (at another veterinary school): chronic dermatitis.
Previous treatment: Ampicillin, unknown dosage, 2 weeks, no effect.
Differential diagnoses:
1. Neoplasia
2. Staphylococcal or mycobacterial infection
3. Deep fungal infection (dog had previously lived in an endemic area for blastomcosis).
4. Ectopic ureters (unlikely given age of onset)
Others?
Diagnostics performed: biopsy for histopathology and bacterial and fungal culture. Histopathology: allergic dermatitis.
Culture: Staphylococcus (pseud)intermedius).
Treatments: Cephalexin 1gm twice daily for one month. No effect.

Owner (not veterinarian) diagnosed food allergy to commercial (human) noodle product (TopRamen®). Fulfilled ‘Koch’s postulate’ by discontinuing product, then when dog’s lesions resolved, challenging with product to reproduce lesions.

Take home messages:
1. When asking owners about diet, include ‘human’ foods, supplements, etc.
2. Owners are sometimes more in-touch with their animal’s diet than the veterinarian.
3. Were the lesions the result of a metabolite that was excreted in the urine, causing a contact allergy?
Ill. ‘Kiki’ 1 year old spayed female DMH

History: licking at ventral abdomen. On intermittent imidacloprid (Advantage®; Bayer) for flea control. Only pet in house. Owners unaffected.

Previous diagnostics: None

Previous treatments: None

Differential diagnoses:
1. Flea allergy dermatitis
2. Infectious or sterile cystitis

Others?

Diagnostics performed: ‘Scotch’ tape prep (negative)

Treatment: Selamectin (Revolution®; Zoetis) once monthly. Recheck after one month: No effect.

Nitenpyram (Capstar™; Novartis) once daily for one month. Recheck: no effect.

Responded to one month on Royal Canin Rabbit and Pea diet.

Take home messages:
1. Food allergy can mimic flea allergy
2. If an animal isn’t responding to the ‘obvious’ treatment, try something else.
3. Was this animal truly pruritic? Or was its food causing gastro-intestinal irritation causing the cat to lick its ventral abdomen?

IV. ‘Brandy’ adult spayed female Springer Spaniel

History: pruritic for at least one year. Licking all four paws but especially the front, biting and caudal 1/3 of body, rubbing periorcically. Only pet in house, owners unaffected.

Previous diagnostics: Skin scrapings (negative), dermatophyte culture (negative).

Previous treatments: unknown

Prednisolone at tapering dose: good response, but unacceptable side effects (polydipsia, polyuria).

Differential diagnoses:
1. atopic dermatitis
2. flea allergy dermatitis (‘no fleas seen’)
3. Sarcoptes scabiei infestation

 Others?

Diagnostics Performed: skin scraping (negative).

Responded in 5 weeks to lamb and rice home-made diet. Eventually maintained on commercial lamb-based dog food.

Take home messages:
1. Food allergy can mimic atopic dermatitis and flea allergy at the same time
2. Even if a home-made diet is used, the pet can often (but not always) be switched to a commercial food.

V. Three cats with facial pruritus

History: These cats were not in the same household – not even in the same state! One lived in Massachusetts, one in Wyoming, and one in California. All presented with pruritus around the face and neck for varying amounts of time. All were the only pet in the house, and owners were unaffected.

Previous diagnostics: skin scraping (3 - negative), biopsy (1): eosinophilic dermatitis.

Previous treatment: Typically all were treated with an antibiotic, some type of flea control, and two received ivermectin. No response. All also received either injectable or oral corticosteroids, which helped to varying degrees.

Differential diagnoses:
1. Notoedres cati infestation
2. atopic dermatitis
3. Otodectes cynotis infestation
4. dermatophytosis

Others?

Diagnostics: skin scraping (3 – negative), fungal culture (3 – negative)

Responded to lamb baby food (2), Purina HA (1) within 1 month (3)

Take home messages:
1. Food allergy is an important differential diagnosis of facial/neck pruritus in cats.
A survey of the histopathological features of skin from the planum nasale and adjacent skin of dogs unaffected by dermatological or respiratory disease

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There is a general belief that cells of the immune system are present in larger numbers in the planum nasale and adjacent haired skin in the dog. However, little published information about the normal histopathological appearance of the skin of this area exists. Biopsies from three sites were obtained from the planum nasale and adjacent haired skin of 25 dogs of varying age, breed and sex, with no evidence of dermatological or respiratory disease. Biopsies were analysed to determine and quantify the immune system cells present in the samples. Slides were stained with haematoxylin and eosin, and toluidine blue; immunohistochemical stains for CD3 and CD79a were applied. There was no evidence of lichenoid inflammation. Immune system cells including lymphocytes and plasma cells were either very rare or present in low numbers. The majority of lymphocytes were of T-cell origin, with only infrequent B-cells identified. Biopsies contained scattered melanophages, consistent with pigmentary incontinence, regardless of the presence or absence of inflammatory cells. Mast cells were present in low numbers; within non-haired skin, superficial mast cells showed close association with the epidermis. In conclusion, immune system cells are not present in large numbers in this anatomical location in clinically normal dogs. Inflammatory change noted in biopsies from this area is therefore likely to be of pathological significance. However, pigmentary incontinence appears to be common at this site, in clinically normal dogs without significant inflammatory cell infiltration, and is therefore not necessarily of pathological significance when seen in isolation in this location.

References


Dermatology disasters: 1) ischemic dermatoses, 2) erythema multiforme, 3) neutrophilic and eosinophilic rare disaster syndromes

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Ischemic dermatoses fall into three categories: dermatomyositis, vaccine- and drug- caused cases, and idiopathic.

Dermatomyositis has been reported primarily in collies and Shetland sheepdogs and their crosses, although other breeds have a sporadic incidence of this disease. As its name implies, the disease affects both the muscles and the skin. Cutaneous changes include crusts, ulceration, vesicles, and/or alopecia around the mucocutaneous junctions, front legs, ear tips, and tail, though other body areas may be affected. Claws may be misshapen. Muscular atrophy may be generalized or may be selective, often affecting the temporal and masseter muscles. Clinical manifestations vary, with some dogs showing only skin or muscular signs, while in others both systems are affected. Serum enzymes such as creatinine phosphokinase (CPK) are usually normal, and muscle involvement often may be proved only by biopsy or electromyography. Skin biopsies generally reveal perifollicular mononuclear inflammation, and occasionally show intracellular edema of the basal cell layer of the epidermis, with subepidermal clefts; advanced cases show a loss of the normal follicular structures. Dermal blood vessels may be decreased in number, be over-distended, smudged, hyalinized and/or sclerotic in appearance. The onset of clinical signs usually occurs before the age of 6 months. The severity of the disease varies greatly, with some dogs improving with age. Females should be spayed as estrus may exacerbate clinical signs. Diagnosis is based on clinical signs and skin biopsy. Recently, the disease in Shetland Sheepdogs has been linked to a change in chromosome.

Drug- or vaccine induced ischemic dermatosis is most commonly associated with rabies vaccination, manifesting itself as alopecia, scale and crusts, and occasionally ulceration, in the area of the vaccine administration, and rarely, in other places as well (especially the pinnal margins and tail tip. Diagnosis is by clinical signs and biopsy. Histology shows involuting, small hair follicles with prominent connective tissue associated with the external root sheaths – the follicles will sometimes disappear permanently. Deep dermal vessels will show a plasmacytic/lymphocytic vasculitis; more superficial vessels will appear as noted above for dermatomyositis.

Idiopathic ischemic dermatosis can occur at any age and any canine breed. Typically, the tail tip, ear margin, face (nasal planum) and sometimes trunk are affected. Claws may be misshapen. Histopathology is as for the vaccine caused type. Recently, there have been anecdotal reports of dogs with this disease having positive Ehrlichia titers and responding completely to doxycyclines – this is probably worth pursuing.

Treatment of ischemic dermatoses
Pentoxifylline (PTX) 10-30 mg/kg q8h, is often effective in controlling ischemic dermatoses. PTX is derived from theobromine. Pentoxifylline, and other methylxanthines produce anti-inflammatory effects. PTX also improves blood flow through narrowed arteries because of the rheological property which allows red blood cells to change shape. It is not known if the improvement in patients with ischemic dermatoses are caused by improved blood flow or via the anti-inflammatory mechanisms. Vomiting is occasionally seen as a side-effect. Eventually some dogs will be able to be tapered down to a q48 hour regimen, or occasionally weaned off the drug.

When PTX is not effective, Atopica® may be used. For small lesions, topical tacrolimus (Protopic®) may be effective. Corticosteroids have had variable efficacy in the author's experience.

Erythema multiforme (EM) is an acute eruption of the skin and mucous membranes. It is characterized in human beings clinically by annular ("target") lesions. While these have been observed in animals, more common signs are mucocutaneous vesicles, ulcers, maculae, and/or urticarial plaques may also be seen. In widespread lesions, the ventrum and peri-ocular areas are often involved. EM may be self-limiting, although by the time the animals arrive at the specialist's office, this is not often the case. EM histologically shows apoptotic (programmed-cell death

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mechanism-activated) keratinocytes, with satellitosis (lymphocytes surrounding the keratinocytes, presumably triggering the programmed cell death mechanism). The incidence/recognition of this disease seems to be increasing. While erythema multiforme has been reported to have an association with drug eruptions, recent work points to TEN and ‘cross-over syndromes’ between the two diseases as more likely to be due to drug involvement. Viruses have also been hypothesized to cause EM in small animals, and there is one report of EM caused by parvo virus in a puppy. The author has seen a few cases that seemed to have an ischemic component, or occurred concurrently with an ischemic dermatitis. While theoretically pentoxifylline should be helpful, the author has seen 2 dogs which had EM induced by pentoxifylline! Intravenous human immunoglobulin has been reported as successful in two dogs when infused on 2 consecutive days (1 g/kg per day). This is a relatively expensive treatment.

Rare Disaster Syndromes
These include 4 rare conditions which are often typified by neutrophils in the skin, and 1 by eosinophils in the skin, but with different underlying causes, and therefore treatments.

Necrotizing fasciitis (‘flesh-eating’ bacteria) caused by Streptococcus canis (biotype 3). Dogs present with fever, swelling, erythema, disproportionate pain on palpation, draining tracts and ulcers. There may be pockets of fluid, usually malodorous. It has been hypothesized that Great Danes and Sharpeis may be predisposed, but too few cases have been reported in the literature to confirm this. There is rapid progression of this disease, as there is of all three diseases in this group. Diagnosis is by clinical signs, skin biopsy and bacterial culture and susceptibility; imaging such as ultrasound, CT scan and/or MRI may be helpful (if available) to find exudates advancing along fascial planes that may not be evident on physical examination. Treatment is widespread surgical debridement and antibiotics – eventually based on susceptibility results, but initially with clindamycin and/or amoxicillin-clavulanate, plus an aminoglycoside. Do not use fluoroquinolones, as these have been associated with possibly engendering or enhancing the extreme toxicity of these Streptococcus strains. NSAIDS should also be avoided, as there is some evidence they may suppress neutrophil activity and mask clinical signs.

Sweet’s (sterile neutrophilic dermatosis) syndrome is a non-infectious, presumed immune mediated condition typified by erythema, fever, malaise, neutrophilia, lameness and neutrophilic effusions into the joints. This is also a rapidly progressive disease, and may be caused by certain medications, as well as arising spontaneously. There is one report of a dog with internal organ involvement. Diagnosis is based on skin biopsy, the lack of bacteria on culture of intact skin lesions or joint aspirates, and response to corticosteroids (prednisolone 1 mg/kg bid initially).

Sterile Pustular Erythroderma of Miniature Schnauzers
A rare, severe, often fatal disease, seemingly limited to miniature Schnauzers, often preceded by bathing. This condition presents with severe depression and malaise, often with fever. Skin lesions are (often dramatic) erythema, pustules or epidermal collarettes, and/or wheals. These dogs are very sick – treatment, when successful, consists of high dose corticosteroids. In a recent abstract, a contact reaction to one of the components in an aloe-based shampoo was implicated.

Staphylococcal Toxic Shock is caused by Staphylococcus sp, presumably S pseudintermedius. Erythema, fever, malaise, and neutrophilia are seen initially; the malaise may be severe and edema of the legs may develop as the disease progresses. This may be the most rapidly progressing of these three diseases. Diagnosis is based on clinical signs – a skin biopsy and bacterial susceptibility should be performed, but any suspicion on the veterinarian’s part for this disease should initiate the immediate use of staphylocidal antibiotics – cephalosporins have been recommended. Pugs may be over-represented.

Well’s like Syndrome is a eosinophilic, generalized dermatitis to cellulitis. It has been associated with either GI signs and/or drugs in some, but not all cases. Peripheral eosinophilia is rare. Skin lesions are papules, macules, and erythema. Pruritus is variable. Histopathology shows an eosinophilic dermatitis, often with eosinophilic ‘flame figures’ (collagen surrounded by eosinophils or their granules). Treatment consists of prednisolone/prednisone at 1-2 mg/kg, then tapered. Treatment duration is variable, but should be continued for at least one month.
References


Cutaneous markers of internal disease

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Feline paraneoplastic alopecia is a ventral alopecia in which the abdominal skin appears to glisten (but is not fragile), may have a secondary Malassezia infection, and has also been associated with dry, fissured foot pads. Necropsy usually reveals an exocrine pancreatic adenocarcinoma, often with metastases to the liver and elsewhere. One cat with a bile duct carcinoma has also been described. Temporary resolution of the skin disease has been reported in one cat which had the primary tumor removed; the lesions recurred following metastases of the tumor. Histology of the skin shows severe atrophy and miniaturization of the hair follicles.

Nodular dermatofibrosis syndrome in German shepherd dogs and occasionally other breeds associated with renal cystadenocarcinomas or cystadenomas has been reported. Histologic study of the nodules reveals dense collagen fibrosis. These nodules are most often found on the distal extremities. Diagnosis of renal lesions is best done by ultrasound. This should be repeated at six month intervals if the disease is suspected but the original ultrasound is normal. While the prognosis is serious, some dogs with benign renal cysts have survived for five years or more after diagnosis. Recent data in the German Shepherd dogs suggest that the syndrome may be caused by a mutation in a previously unidentified tumor suppressor gene.

Thymomas have been associated with an exfoliative dermatitis described in older (often ‘orange’) cats. The exact mechanism is unknown, but an erythema multiforme-type reaction has been proposed. If detected, the tumor’s removal will lead to resolution of signs. Recently, radiotherapy has been reported as a therapeutic option.

Superficial necrolytic dermatitis

Also known as SND, hepatocutaneous syndrome, epidermal metabolic necrosis, or diabetic dermatosis, this disease is being seen with increased frequency in dogs, and has also been reported in the cat and a red fox (Vulpes vulpes). The cutaneous lesions include crusting, erythema, exudation, and alopecia periorally and periocularly, around the genitals, and the distal extremities, as well as hyperkeratosis and ulceration of the footpads. The skin disease may precede the onset of the signs of the internal disease. Histopathologic findings include superficial perivascular-to-lichenoid dermatitis, with marked diffuse parakeratotic hyperkeratosis and striking inter- and intracellular edema limited to the upper half of the epidermis (‘red, white and blue sign’). Diagnosis is usually made by clinical signs, confirmatory histopathology, and an ultra-sound finding of both hyper- and hypo-echoic areas in the liver (‘Swiss-cheese’ or ‘honey comb’ pattern).

Superficial necrolytic dermatitis resembles the glucagonoma syndrome (necrolytic migratory erythema) of humans, which is usually associated with hyperglucagonemia and a glucagon-secreting alpha-cell neoplasm of the pancreas. Hyperglucagonemia has also been documented in dogs with this syndrome; however, dogs tend to have hepatic parenchymal damage much more commonly than gluconomas. Dogs with SND have profoundly low levels of plasma amino acids.

Therapy is best effected with the infusion of amino acids (ex: Amnosyn®) given intravenously in a central vein, at an approximate rate of 60-80 mg/kg/24 hr. Osmalality and/or neurologic signs should be monitored (although problems are relatively uncommon). This is often performed on a daily basis for 2-3 days, and may need to be repeated on a q 3-6 week basis.

An alternative is the use of oral medications: ProCel® Powder (1 scoop/5kg q12h; Global Health Products www.globalhp.com 1-800-638-2879) or whey protein powders, scrambled eggs (?/day), elemental Zn (2 mg/kg/ day), and sAME (or similar liver protectants).
When the underlying disease can be treated (drug-induced hepatopathy, removal of glucagonoma) and secondary skin infections (bacterial and/or yeast) are treated, these dogs have usually responded well for variable lengths of time, sometimes for more than one year.

**Metastatic Pulmonary Carcinomas**
This problem has been reported in cats: the lesions occur on the distal extremities, especially the front feet, and look more like inflammatory pododermatitis than a neoplastic process. Their presence may be noted before pulmonary signs are noted. Rarely, the neoplasm may metastasize to other areas on the body, such as the abdominal skin. The neoplasm may be either a bronchogenic or squamous cell carcinoma. Palliative treatment to reduce the discomfort may be attempted (topical or systemic corticosteroids to reduce edema, etc).

**Endocrine Disease**
Hypothyroidism, hyperadrenocorticism, and testicular neoplasia (particularly Sertoli cell tumor) may all cause cutaneous signs. Only their presentations will be covered.

**Hyperadrenocorticism**
Dermatologic signs noted in dogs with hyperadrenocorticism but not limited to that disease include:
1. Nonpruritic alopecia
2. Nonpruritic hyperpigmentation
3. Recurrent pyoderma
4. Recurrent dermatophytosis or demodicosis
5. ‘Seborrhea’ (excess scale, crust, etc)
6. Easily bruised skin

More specific clinical signs are:
1. Comedones
2. Calciosis cutis
3. Thin skin
4. Perianal adenomas and hypertestosteronemia in females or neutered males

In one five year retrospective study, clinical features of hyperadrenocorticism were evaluated in 60 dogs that had cutaneous lesions as the first noticed clinical signs. Diagnosis was made by the ACTH stimulation test, the low dose dexamethasone suppression test, the high dose dexamethasone suppression test and/or the plasma ACTH concentrations. The final diagnoses were 58 dogs with pituitary dependent hyperadrenocorticism, and two dogs with adrenal neoplasia. In retrospect, some of these dogs may have had non-classic adrenal disease (‘adrenal sex-hormone’ abnormalities), although only 5 of the 60 dogs (8%) were a breed typical of this syndrome (all were miniature poodles). Interestingly, two dogs were related (mother/son). Similar reports of related dogs have been reported.

While not referenced against the population at large, the most common breeds were poodles, dachshunds and Llasa Apsos. Other breeds reported to be at risk are boxers and Boston terriers.

The most common cutaneous lesions were truncal alopecia (62%), pyoderma (55%) and hyperpigmentation (43%). Less common dermatologic manifestations were thin skin (14%), non-truncal (face, head) alopecia (13%), *Demodex canis* infestation (8%), and comedones (5%); only one had calcinosis cutis. Pruritus, seen in 15% of the dogs, was always associated with pyoderma, seborrhea, demodicosis, and/or calcinosis cutis.

**Hypothyroidism**
Dermatological features occur in at least 80 per cent of cases. The classical description is of bilaterally symmetrical, non-pruritic alopecia which spares the distal limbs, with varying degrees of hyperpigmentation and thickened, non-pitting skin (myxoedema). In fact, myxedema is uncommon, while the alopecia, with or without hyperpigmentation, may be focal, multifocal, regional or generalized and asymmetrical. The tail may lose most of its hair (‘rat-tail’), as may the dorsal muzzle. The haircoat may be easily epilated and may fail to regrow or regrow only slowly after clipping. In some breeds, particularly the Boxer and the Irish Setter, the hair may not be shed resulting in hypertrichosis (‘carpet-coat’). In the Golden Retriever and the Irish Setter, the ‘feathers’ on the legs may become a yellow colour (‘blond frizzies’). Seborrhea is relatively common and may be dry, greasy or inflamed. Comedones may be present, particularly over the dorsum. Hyperkeratotic plaques may occur around the borders of the pinnae. Bacterial folliculitis occurs frequently. In the presence of seborrhea or pyoderma, hypothyroidism may be accompanied by pruritus.
Sertoli cell tumor

Clinical signs: Nonpruritic alopecia
Nonpruritic hyperpigmentation
Enlarged nipples and/or mammary glands
Palpable mass in testicle (NOT always present)
Linear preputial dermatosis (may also be seen in any dog receiving estrogen treatment.)
Occasionally, severe papular, pruritic rash
Thrombocytopenia, anaemia may be present (petechia)

References

Update on atopic dermatitis and its secondary infections

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Etiology

Atopic dermatitis is the arguably the second most frequent allergy in dogs (after fleas) and the third most frequent allergy (after fleas and food) in cats. There is a strong genetic tendency in dogs, with golden retrievers, Labrador retrievers, terriers, Dalmatians, and Shar-Peis among the predisposed breeds. At the University of California at Davis (UCD), breeds at greatest risk for atopic dermatitis are Golden Retriever, West Highland White Terrier, Chinese Shar-Pei, Bull Terrier, Bichon Frisé, and the Tibetan Terrier. At Colorado State University (CSU), the three most common groups of breeds examined with atopic dermatitis are retrievers, terriers and spaniels. In the UK, atopic dogs were more likely to have atopic offspring - this was particularly evident for atopic sires. Interestingly, in a large study of West Highland White Terriers, Boxers, and Bullterriers in Sweden, feeding a diet that was at least partially home-cooked to the dam was associated with a lower incidence of atopic dermatitis in the offspring. A genetic tendency has not been well-documented in cats, but is suspected.

Atopic dermatitis is an allergic response, mediated by IgE. The allergens gain entrance to the body by either the respiratory or percutaneous route, binding to antibodies in the skin which are themselves bound to mast cells. This antibody-allergen binding causes the mast cells to release various inflammatory substances (histamine, etc.). Sensitization is probably an interaction of the Langerhans’ cells in the epidermis, which bind the antigen and travel to the regional lymph nodes, and both T and B lymphocytes. It would seem likely that, as in people and mice, the subclass \( T_{H-2} \) is responsible for promoting B lymphocyte overproduction of antibody when the Langerhans’ cell presents the antigen. It has been recently shown that CD4+ T cells ("helper cells") predominate in the skin of atopic dogs (as they do in people) over CD8+ T cells ("suppressor cells") although both populations are increased in atopic dogs' skin compared to that of healthy dogs. In a recent article, atopic dogs were shown to have much higher serum values of IgG1 than other dogs.

The barrier function of the epidermis is defective in atopic dogs. A recent article demonstrated that atopic dogs have abnormal (inadequate) lipid interstitium in the stratum corneum, and recent work suggests that some atopic dogs may have a defect in filaggrin, an important component of the stratum corneum (the outermost, 'horny' layer of the epidermis); this may translate into more susceptibility to both allergen and infective organism access to the skin.

Clinical Presentation

Atopic dermatitis may be seasonal or year-round. Dogs most commonly start showing signs between one to seven years of age. In a study at UCD, the average age of affected dogs was 1.66 years, with 95% of the dogs first showing clinical signs some time less than 5 years of age. "Exotic breeds", such as the Akita, Shar-Pei and Chow Chow, may show signs as young as six months. Initially, the season may be quite short, but as (presumably) the dog becomes allergic to more allergens, year-round pruritus frequently results. Lesions in dogs are generally found on the feet, ears, axilla, and face, with conjunctivitis present more frequently than is commonly reported. Pruritus, typified by erythema, alopecia and hyperpigmentation is the most frequent sign. Clinical signs may become generalized in severe cases.

In cats, lesions are generally found on the face, rarely on the feet, and are occasionally generalized. Pruritus, typified by erythema, alopecia and hyperpigmentation is the most frequent sign. Miliary dermatitis and the eosinophilic granuloma complex have also been reported as being secondary to atopic dermatitis in some cases. Age of onset, or sex predilections have not been reported in cats, although Himalayans, Abyssinians, and Persians had a predilection in one study.

Atopic dogs are prone to secondary infections with \textit{Staphylococcus pseudintermedius} and \textit{Malassezia pachydermatis}. Thus, clinical signs of superficial pyoderma (epidermal collarettes, papules), or yeast infection (waxy brown exudate on skin or proximal claws, erythema interdigitally or under tail) are common. \textit{S pseudintermedius} may further exacerbate the atopic state by eliciting production of IgE specific for \textit{Staphylococcus}, as well as by producing staphylococcal protein A (SPA), which may nonspecifically bind to IgE molecules on mast cells. In return,
the atopic state may contribute to or enhance pyoderma by the pruritus physically reducing the barrier the stratum corneum poses for infection, by the increased ability of *S. pseudintermedius* to "stick" to atopic dogs’ corneocytes, or by the degranulation of mast cells making the epidermis more permeable to staphylococcal antigens. In addition, atopic dogs’ carriage of *S. pseudintermedius* is greater than healthy dogs. While *S. pseudintermedius* is the most commonly isolated bacteria from superficial pyodermas in dogs, *S. aureus* and *S. schleiferi* have also been isolated. Diagnosis of superficial pyoderma is usually made by clinical signs; diagnosis of *Malassezia* infections is made by scrape or tape preparations of the superficial skin, stained with DiffQwik™ and examined under oil immersion. Atopic cats may be prone to secondary infections with *S. pseudintermedius* and *Malassezia pachydermatis*, although seemingly not at the same rate as dogs.

It is vital to treat secondary bacterial infections (usually superficial pyodermas), as in most animals these are also contributing to pruritus. The author uses the following antibiotics:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Dosage</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>Cephalexin</td>
<td>20-30 mg/kg q 8-12 h</td>
<td></td>
</tr>
<tr>
<td>Cefpodoxime (Simplicef™)</td>
<td>5-10 mg/kg q 24 h</td>
<td></td>
</tr>
<tr>
<td>Lincomycin (Lincocin ®)</td>
<td>20 mg/kg q 12 h</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>30 mg/kg q24h</td>
<td></td>
</tr>
<tr>
<td>Enrofloxacin (Baytril®)</td>
<td>5-10 mg/kg q 24 h</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-Clavulanate (Clavamox®)</td>
<td>13.75 mg/kg q 12 h</td>
<td></td>
</tr>
<tr>
<td>Marbofloxacin (Zeniquin®)</td>
<td>3- 6 mg/kg q 24 h</td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td>10 mg/kg, q24h, 4 days/week</td>
<td></td>
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<tr>
<td>Doxycline</td>
<td>5 mg/kg q12h</td>
<td></td>
</tr>
<tr>
<td>TMS</td>
<td>30 mg/kg q12h</td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>11 mg/kg q12h</td>
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</table>

With the increase in methicillin-resistant *S. pseudintermedius*, *S. aureus*, and *S. schleiferi*, the author now strongly recommends culture of any dog with epidermal collarettes that fails to begin to respond to one of the above antibiotics over a 3-4 week period of time. Epidermal collarettes may be cultured using a dry sterile culturette rolled across the collarettes. It should be pointed out that while methicillin-resistant *S. pseudintermedius* infections are of course more difficult to treat, they are not more virulent or visually –striking than those due to methicillin-susceptible *S. pseudintermedius*. Previous (i.e. within the last year) hospitalization, surgery, or previous antibiotic treatment are all possible risk factors for the development of methicillin-resistant *S. pseudintermedius* infections.

Shampoos may be helpful as adjunct treatment in pyoderma, particularly in superficial pyodermas. There are many good, effective anti-bacterial shampoos available. The author’s favorite is an ethyl-lactate containing shampoo (Eldermer®, Virbac), which is effective, lathers well, and is well-liked by owners. A 4% chlorhexidine – tris-EDTA shampoo has also shown very good results (Triz-chlor 4®, Dechra). Interestingly, miconazole, an anti-fungal molecule, also has efficacy against *Staphylococcus* sp.

For treatment of *Malassezia*, ketoconazole (5mg/kg q24h), itraconazole (5mg/kg q24h) or fluconazole (5 mg/kg q24h) have all been effective in the author’s hands. Terbinafine (30 mg/kg q24h) may be used in dogs that do not tolerate azoles. Topicals such as Malaseb® (DVM: chlorhexidine and miconazole), Mal-A-Ket® (Dechra: boric and acetic acids, and ketoconazole) or Resizole® (Virbac: miconazole), are helpful. ‘Wipes’, such as Malaseb®, Malacetic®, Triz-Chlor 4® or Duoxo® pads are VERY useful in the treatment of interdigital yeast and bacterial infections.

**Diagnosis**

Atopic dermatitis is diagnosed by history, physical examination, and (except in the “classic” case of recurrent, strictly seasonal "typical" clinical signs, erythema/pruritus of face, etc.) ruling out other causes of pruritus, especially food allergy, ectoparasites, etc. Use of intradermal skin testing or serologic tests, looking for allergen-specific IgE, should only be performed if the owners are interested in hyposensitization. Because of the high incidence of “false positive” (or, at least, clinically non-relevant) findings, these tests should only be performed if other diagnoses have been ruled out.

There is a great deal of controversy over which is the more accurate, intradermal skin testing or serologic tests. In three studies, at UCD, CSU and Gifu University (Japan) involving a combination of over 250 dogs, the number of positive responses to hyposensitization were similar regardless of whether the hyposensitization solutions were based on IDST results or ELISA tests.
Treatment

The most appropriate treatment is usually hyposensitization injections, which are effective in about 65% of the dogs treated at UCD, and 70-80% of the cats in the author’s experience. Hyposensitization requires a tremendous amount of clinician-client communication, especially so that the owner knows:

1. Hyposensitization should be continued for at least 1 year before final evaluation of efficacy.
2. If effective, the treatment will almost certainly need to be continued for the animal’s life (in our study at UCD, only 2 dogs out of over 150 had their hyposensitization discontinued without a recurrence in the following 2 years).
3. If effective, the animal may still need ancillary treatment (antibiotics, antihistamines, fatty acids, etc.).
4. Minor side effects (pain at the site of injection) and major side effects (anaphylaxis - very rare).
5. Expense (in the USA): around $1000 per year, depending upon frequency of injections (most dogs receive a maintenance injection q7-10 days) and need for a veterinary examination due to pruritus or minor side effects.
6. A newer method of hyposensitization, oral or SLIT (Sub-lingual Immunotherapy) requires consistent twice daily administration.

More recently, oral hyposensitization (SLIT: SubLingual ImmunoTherapy) has been used. Our clinical impression at UC Davis is that perhaps as high as 50% of dogs that do not respond to the hyposensitization injections may improve on the oral therapy. As time goes on, we suspect the opposite may also be true.

There is some evidence that mold allergens should not be mixed in the same solution as pollens. Possibly because the molds have proteases that degrade the antigenicity of the pollen proteins. A recent article confirmed early research that allergen solutions for hyposensitization must be customized to the individual dog (i.e. one cannot just use a ‘regional mix’).

If hyposensitization fails or if the owner declines this therapy, antipruritic drugs may be necessary. Prednisolone (1 mg/kg once daily [double for cats], then tapered to the lowest every other day dosage needed) is usually effective. Triamcinolone (3-4 mg/cat) or dexamethasone (0.1 mg/kg daily) then taper; these medicaitions may be used with caution in cats, but are very ‘Cushing-ogenic’ in dogs.

Antihistamines commonly used in dogs are cetirizine (1mg/kg BID), diphenhydramine (2.2 mg/kg TID), hydroxyzine pamoate (2.2 mg/kg TID), or chlorpheniramine (0.2-0.8 mg/kg BID to TID); in one study diphenhydramine and hydroxyzine were both the most commonly used antihistamines and the most frequently effective. Note that cetirizine is the active metabolite of hydroxyzine, so if one doesn’t work, the other probably won’t either. Clemastine has very low bioavailability in the dog when given orally, and cannot be recommended. In cats, chlorpheniramine (2-4 mg/5 kg BID) or clemastine (0.1 mg/kg BID) may be used. Cetirizine has been shown to be ineffective as a non-pruritic agent.

Amitryptiline, a tricyclic antidepressant is sometimes helpful in controlling pruritus. In dogs, the dose is 2.2 mg/kg BID. In cats, the author has used 5-10 mg/cat BID.

A combination medication of trimeprazine 5mg and prednisolone 2 mg (Temeril-P® [Vanectyl-P® in Canada], Pfizer) has been quite helpful in reducing the amount of prednisone which must be given to atopic dogs. The drug is given at an empiric dose of 1 tablet BID for dogs less 10 kg, 2 tablets BID for dogs between 10 and 25 kg, and 3 tablets BID for dogs over 25 kg.

Products containing essential fatty acids (EFAs: omega-3 and omega-6)) have been used as nonsteroidal antipruritics. These drugs apparently interfere with the production and metabolism of arachidonic acid and other "pro-inflammatory" substances. Most dogs in western countries are ingesting adequate amounts of omega-6 fatty acids, thus more likely necessitating supplementation with omega-3 fatty acids, approximately 66mg/kg of a combination of eicosapentaenoic acid & docosahexaenoic acid. In dogs, these may have as high as a 25% chance of reducing pruritus, particularly when combined with antihistamine treatment. When EFA supplements are included in the dog food, the success rate in one open trial was 42% (good to excellent control of pruritus); in another trial it was 44%. In this latter trial, dogs responding to the test diet had a different pattern of fatty acid change in the plasma and the skin as compared to the dogs which failed to respond to the diet, suggesting that there are subsets of atopic dogs with different fatty acid metabolism capabilities. Somewhat in contrast, another article
noted that the improvement seen in atopic dogs with EFA supplementation did not seem to be correlated with total fatty acid intake or with the ratio of omega-6:3 fatty acids. A very well written report documented the steroid-sparing effect of EFAs in some atopic dogs. Another report documented some beneficial effect in the use of dog foods enriched with EFAs while a recent report documented improvement in pruritus when atopic dogs were fed certain diets that were recommended for allergic dogs.

Antihistamines in conjunction with essential fatty acids may give relief from clinical signs in as high as 50% of atopic cats.

Cyclosporine is available as Atopica® (Novartis) in 10, 25, 50 and 100 mg capsule sizes. While in people this medication should be given on an empty stomach, one article showed that concurrent food administration does not influence the clinical response to the drug. It is well-tolerated in most dogs. Because low doses are used for treating atopic dermatitis, usually 5-7 mg/kg/day or less, adverse effects are uncommon. The most common problem is nausea and loss of appetite. This may be overcome in many cases by keeping the product frozen until administered, or by giving maropitant (Cerenia®, Zoetis). Because of expense, particularly in large breed dogs, administration concurrently of ketoconazole will enable a reduction of Atopica® dosage. (This is due to the body’s metabolism of both drugs). In general, with a dose of ketoconazole of 5 mg/kg, the author has seen good results using only 50% of the canine dose of cyclosporine (2.5 mg/kg per day instead of 5 mg/kg per day). One article suggests that as many as >35% of atopic dogs treated with cyclosporine for 4 months may not relapse for as long as 40 days after cessation of the cyclosporine. Cats treated with the liquid preparation of Atopica have a similar effective decrease in their pruritus. The drug is reasonably well tolerated by cats. While any cat receiving Atopica should be kept indoors to limit the possibility of contracting toxoplasmosis, this is especially important with cats that have no IgG antibodies to the parasite, or (presumably) have only IgM antibodies (indicative of an active infection).

Apoquel® (oclacitinib – Zoetis) a Janus-kinase-1 inhibitor that interferes with the effectiveness of interleukin 31 (a mediator of pruritus at the nerve level) has just become available in the USA. In clinical trials, it has been well-tolerated by dogs and suppresses pruritus rapidly (within 24 hours). Dosages approximated to 0.4-0.6 mg/kg orally twice daily for 2 weeks, then decreased to once daily. The author has found that perhaps as high as 30% of dogs will become pruritic after decreasing the dosage to once daily, although typically not as pruritic as before the medication was started. The drug is contraindicated in dogs with a history of demodicosis or neoplasia. Adverse effects are rare – gastrointestinal upset and lethargy have been noted, and rare elevations of liver enzymes may occur.

Topical products:
Oatmeal-based shampoos, or shampoos containing 0.5-1.0% hydrocortisone, are also helpful adjunctive therapies in the dog (or rare cat that enjoys a bath). Ideally, they should be used at least two to three times per week. The introduction of “Resi” products, basically rinses designed to be left on the pet, have given encouraging results. Chief among these is Resi-Cort™ (Virbac) a 1% hydrocortisone product, which may be used once to twice weekly.

Genesis™ Spray (VIRBAC). The active ingredient is 0.015 % triamcinolone acetonide. While not optimal for long term management, this spray has a low risk of corticosteroid side-effects if used on problem areas (especially the feet) to ‘cool down’ the pruritic response.

Duoxocalm® shampoo, spot-on and spray (Sogeval). These products contain phytosphingosine, a substance that may normalize the stratum corneum in atopic dogs, as well as hinokitol, a plant derived substance with anti-infective properties. It is now the widest used non-steroid anti-pruritic shampoo in France, according to Sogeval. The Duoxocalm® Seborrhea Spot-on may also be effective in this regard.

Dermascent® (LDCA) another topical spot-on with omega-3 and -6 fatty acids which claims to be effective in restoring the barrier function of the epidermis in atopic dogs.


Cyclosporin concentrations in dogs and cats 20-24 hours post dosing

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Introduction
Cyclosporin (CsA) is a drug with low bioavailability in both dogs and cats.\(^{1,2}\) Such low bioavailability can lead to significant interindividual variation in drug exposure between individuals.\(^{3}\) Significant side effects have been uncommonly associated with CsA use, including opportunistic infections and diabetes mellitus.\(^{4-6}\) While consensus is lacking, there is evidence to suggest that high serum concentrations of cyclosporin may increase the risk of these side effects.\(^{4,5,7}\) Because of the low bioavailability of CsA, these concentrations may be achievable unexpectedly in some individuals at recommended doses.\(^{5}\)

The aim of this study was to examine 20 to 24 hour trough concentrations (C\(_{20-24}\)) of CsA in canine and feline patients of a specialist referral centre and compare the results with published pharmacokinetic parameters.

Methods
Patient records at the Melbourne Veterinary Specialist Centre were searched from 1st January 2012 to 15th March 2015, once using the keyword 'trough' and once with 'cyclo' or 'ciclo'. The two lists were correlated and the resulting records manually searched for patients who had been treated with cyclosporin continuously daily for at least 2 weeks before blood sampling (EDTA tube) for assay of whole blood cyclosporin concentrations.

Exclusion criteria included concurrent use of medications which may have affected CsA concentrations, concurrent disease (primarily liver disease) that may have affected CsA concentrations, any CsA assay aside from CEDIA PLUS (the assay used by most but not all Australian commercial laboratories), blood sampling taken less than 20 hours or greater than 24 hours after the previous cyclosporin dose and use of any therapeutic CsA that was not either Atopica or Neoral.

For included cases, the name, patient number, species, breed, age, sex, disease requiring CsA therapy, form of CsA used, dose(s) and CsA C\(_{24}\) were recorded. Patients were then grouped to species, and whether CsA C\(_{20-24}\) was <500ng/ml, 500-999ng/ml or ≥1000ng/ml. Breed, age, sex, form and dose of CsA were examined to see if there was any difference between the groups. Lastly the mean and standard error of the initial CsA C\(_{20-24}\) from each of the cat and dog groups were compared with published references.

Results
Thirty-nine cats were included in the study. Of these, CsA C\(_{24}\) was <500ng/ml in 12/39 cats, 500-999ng/ml in 14/39 cats and ≥1000ng/ml in 13/39 cats. No obvious differences were noted in sex, breed, age, form of CsA or mean CsA dose between the groups. The mean CsA C\(_{20-24}\) of the feline group was 937.3 ng/ml (SEM 121.2ng/ml). The published mean CsA C\(_{20-24}\) (using HPLC assay and 8mg/kg CsA dosing) is approximately 242ng/ml (SEM ~73ng/ml).\(^{10}\)

Forty-three dogs were included in the study. Of these, CsA C\(_{24}\) was <500ng/ml in 41/43 dogs, 500-999ng/ml in 1/43 dogs and ≥1000ng/ml in 1/43 dogs. No obvious differences were noted in sex, age, form of CsA or mean CsA dose between the groups. Both of the dogs with higher CsA C\(_{20-24}\) >500ng/ml were West Highland White Terriers (WHWT) of which there were five in total in the study. This outcome was significantly more than expected for this breed (Fisher’s exact test, two-tailed P value=0.0486).\(^{9}\)

The mean CsA C\(_{20-24}\) of the canine group was 134.58 ng/ml (SEM 27.8ng/ml). The published mean CsA C\(_{20-24}\) (using FPIA assay converted to HPLC equivalent and 5mg/kg CsA dosing) is approximately 38.4ng/ml (range of expected concentration in 90% of the population 13.2-63.6 ng/ml) in a laboratory beagle model and 80.68mg/ml (SEM 14.63ng/ml; range to 0~242ng/ml, mean dose 4.6mg/kg CsA) in client owned dogs.\(^2\)
Discussion

While the mean CsA C\textsubscript{20-24} in dogs was somewhat higher than the published data it was still relatively low. In contrast, the feline mean CsA C\textsubscript{20-24} was markedly higher than predicted by the published pharmacokinetic data. The difference in CEDIA plus to HPLC would normally be expected to account for less than 10-15% of this using human data.\textsuperscript{10a} There are several possibilities that may explain this. Firstly, cats may have a higher frequency of heterophile antibodies that can interfere with the CEDIA test.\textsuperscript{11} However a linear reduction in trough proportional to dose reduction in ten cats where repeated CsA C\textsubscript{20-24} was performed (data not shown) would suggest this is not the case. Secondly, cats may metabolise cyclosporin differently to dogs and humans with higher production of crossreactive AM1 (M17) or AM9 (M1) active metabolites,\textsuperscript{11a} or AM4n (M21) or AM19 (M8) inactive metabolites\textsuperscript{11a} which may be measured on a CEDIA assay leading to falsely elevated results compared with HPLC assay which measures the parent compound alone.\textsuperscript{12} Further study directly comparing the two assays using feline blood is required to examine this possibility. Lastly, it may be that the clinical population of cats, similar to that in dogs, shows more variability in absorption and metabolism of cyclosporin compared with the published laboratory population.

Of note is one cat in this study who initially tested <500ng/ml but after many months of therapy showed reduced appetite and weight loss. Subsequent testing showed CsA C\textsubscript{20-24} of >2000ng/ml. The cause for this was unknown. Given the proposed role of the intestine as well as the liver in the first pass effect of CsA in humans it is not inconceivable that intestinal as well as liver changes may lead to increased CsA C\textsubscript{20-24}.

WHWT have been prominent in some reports of opportunistic infections (including \textit{Burkholderia cepacia} deep pyoderma, nocardiosis) with the latter reporting one case with elevated trough concentrations of 1780ng/ml in combination with ketoconazole.\textsuperscript{4,6} In addition, treatment of WHWT with CsA in the UK and Europe appears to be, based on pharmacovigilance data, associated with an increased incidence of diabetes mellitus (DM), which is otherwise rare, and is now listed on the European Medicines Agency Summary of Product Characteristics.\textsuperscript{8} WHWT were overrepresented in the higher trough concentration groups in this study. It should be considered that WHWT may have a pharmacogenetic interaction with CsA leading to an increased risk of abnormally high drug exposure and subsequent adverse reactions. Further study is needed to clarify the risk in this breed.

There were a couple more limitations in this study.

Firstly, the incidence of health events that may have been associated with cyclosporin administration in this case series was not specifically studied. Further examination of this may reveal more associations between CsA C\textsubscript{20-24} and adverse health events.

Secondly, there are questions as to whether CsA C\textsubscript{20-24} is the most appropriate measurement to correlate with drug exposure. In humans it has been suggested that CsA C\textsubscript{2} to measure C\textsubscript{max} is correlated better to the AUC and thus prediction of clinical outcomes and adverse reactions.\textsuperscript{15} This has not been assessed in dogs, and in cats there is conflicting data as to whether CsA C\textsubscript{2} is proportional to the AUC (and thus total drug exposure).\textsuperscript{1,10} Given the data associating high trough concentrations with clinical toxoplasmosis in cats\textsuperscript{5} it seems prudent for now to continue to recommend CsA C\textsubscript{20-24} as the optimal time of CsA measurement. This recommendation may change in the face of new data.

Conclusion

While there remains much debate over the significance of CsA C\textsubscript{20-24} and the prevalence of adverse reactions, there remain several reports suggesting that high trough concentrations (700-1000 ng/ml in cats\textsuperscript{5,16} and 400-600 or more in dogs\textsuperscript{4,17-19} may be frequently associated with adverse reactions. Based on current information the following recommendations are thus made for CsA monitoring:

Cats
- Commence therapy at ~5mg/kg q24h rather than 7mg/kg as recommended
- Perform CsA C\textsubscript{20-24} in all cats 1-2 weeks after starting (even for cats going onto planned short courses e.g. 6-8 weeks)
- Warn clients that there about 2/3 of cats will likely need a second trough concentration.
- If CsA C\textsubscript{20-24} is >600 aim to taper the dose to target 500 or less. If a dose reduction is needed there appears to near first order pharmacokinetics (data not shown) (e.g. if the goal is <500 and CsA C\textsubscript{20-24} is 2000ng/ml then decrease the daily dose to ¼ of the previous dose then repeat trough after 1-2 weeks).
- Repeat CsA C\textsubscript{20-24} if any sign of a possible adverse health event that may be associated with the CsA, or concurrent administration of a drug that may affect the cyclosporin concentration (e.g. fluconazole)

**Dogs**
- Perform CsA C\textsubscript{20-24} in all WHWT 1-2 weeks after starting (even for dogs going onto planned short courses e.g. 6-8 weeks)
- Routine CsA C\textsubscript{20-24} is recommended but optional in other breeds
- Warn clients that there about 40% of WHWT will likely need a second trough concentration. It is unlikely in other breeds.
- If CsA C\textsubscript{20-24} is >600 aim to taper the dose to target 500 or less. If a dose reduction is needed there appears to near first order pharmacokinetics (data not shown) (e.g. if the goal is <500 and CsA C\textsubscript{20-24} is 2000ng/ml then decrease the dose to ¼ of the previous dose then repeat trough after 1-2 weeks).
- Perform CsA C\textsubscript{20-24} in any breed if any sign of a possible adverse health event that may be associated with the CsA, or concurrent administration of a drug that may affect the cyclosporin concentration (e.g. fluconazole)

**References**


Can some contact allergies be treated with immunotherapy in dogs? Food for thought.

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Introduction
Atopic dermatitis is a common problem in dogs and allergen-specific immunotherapy (ASIT) is reported to lead to at least 50% improvement in clinical signs in 50-100% of cases.\(^1\) IgE-mediated contact allergy has been reported secondary to several grasses (paspalum \([Paspalum notatum]\), kikuyu \([Pennisetum clandestinum]\) and Queensland blue couch \([Digitaria didactyla]\)\(^2\) and a ground cover \((Callisia fragrens)\).\(^3\) Mason & Ruutu\(^2\) noted that on Western blots of atopic dog serum, allergens in the pollens of grass and weeds using in intradermal testing did not overlap with allergens from extracts of the same plants. However, in data presented, in contrast to the proceedings notes, some cases also showed IgE-mediated reactions to similarly sized proteins in both the pollen and the leaves of the grass. Use of ASIT to treat avoidable botanical contact allergens has not been previously reported in dogs. This paper reports two cases of concurrent atopic dermatitis and avoidable botanical contact dermatitis showing significant improvement on ASIT.

Case Reports
Case 1
2yo FS bulldog with a long history of unilateral otitis, and irritated and swollen feet both dorsally and ventrally from about 16 months of age. It had been noted to be worse on wet or cut grass. Artificial grass was the only grass in the yard at home. At presentation to ASES there was mild erythema and scaling in the flexor carpal and tarsal regions, papules on the medial pinnae and moderate erythema and hyperhidrosis in dorsal and ventral interdigital regions. Cytology revealed mixed bacterial and yeast infection in the ears and interdigitally. These were treated with systemic antibiotics and antifungals, and following an ear flush under anaesthesia, topical enrofloxacin, dexamethasone and ketoconazole in plurionic gel depot topical ear medication.

Intradermal testing at this time showed moderate or strong reactions to moth, American cockroach, \(Dermanyssus farinae\), \(D. pteronyssinus\) and \(Tyrophagus putrescentiae\). Allergen-specific IgE testing (HEKSA) showed positive reactions to all pollens except dock, sorrel, Yorkshire fog, Johnson grass and cypress tree in addition to \(D. farinae\), \(D. pteronyssinus\), \(T. putrescentiae\), cockroach and ant.

Following infection resolution there remains a moderate dry erythema on the ventral interdigital and flexor carpal and tarsal regions. Tapering prednisolone administration revealed the lowest comfortable dose 0.35mg/kg eod though skin lesions were still visible interdentally. A subsequent 5 day plant avoidance trial done off prednisolone revealed a complete improvement in residual paw lesions and pruritus, with rapid relapse of erythema and licking with re-exposure. ASIT was commenced and boots were being used to minimise skin flares when outside the yard at this stage. 9 months later there was no avoidance wet grass required and no problems with occasional irritation and hyperhidrosis noted on only the dorsal paws at recheck with no ventral erythema. Boots were not being used and the prednisolone dose was 0.31mg/kg twice weekly.

Case 2
2yo Staffordshire bull terrier with a history of pruritus on the ventrum, axillae, paws and legs since 4mths of age (commencing in summer). The problem was initially episodic with five visits to the referring veterinarian in the first 12 months of skin problems, but more continuous in the 9 months prior to referral, and was noted to be exacerbated by grass contact and this was made apparent with significant improvement when the dog was kept inside with minimal skin issue post patellar surgery at 18 months of age. Occasional flares of eye and ear inflammation had also previously been noted. At presentation to ASES there was moderate to marked papular erythema on the ventrum, caudal thighs, perineum, flexural carpal and tarsal regions, ventral interdigital regions and pinnae. Cytology revealed a mixed otitis and pyoderma. The skin and ear infections were treated with clindamycin 5.5mg/kg q12h and topical ear medication (Easotic ®; Virbac) daily.
A 6 day plant avoidance trial at home resulted in a significant partial reduction in pruritus and complete resolution of skin erythema. Within nine hours of grass rechallenge, skin lesions started to return with a marked worsening especially of pedal pruritus. Subsequent intradermal testing showed strong reactions to all grass pollens and moderate to strong reactions in 23/39 other pollens, in addition to a single strong reaction to ant. A diagnosis of concurrent contact allergy and atopic dermatitis was made.

ASIT was commenced in June with a prednisolone requirement of 0.2-0.3mg/kg daily. 17 months later the dog was comfortable with a prednisolone dose of 0.2mg/kg every 2-3 days in association with ASIT. Occasional bouts of itch and yeast infection were still noted but no flares were noted with grass contact.

Discussion

Both these cases showed history and clinical signs consistent with both ‘inhaled atopy’ and ‘contact atopy’ as per Mason and Ruutu including a short haired coat, involvement of the flexor carpus / tarsus and popular dermatitis. Both cases showed significant improvement in clinical signs with a 5 days exclusion trial from plant contact in the same environment, and re-exacerbation in a short period following re-exposure. Following immunotherapy both dogs showed significant improvement in clinical signs and were able to tolerate grass exposure without allergic flare.

It should be noted though there are limitations to these cases including that

- the cause for improvement off the grass and worsening on re-exposure was not isolated. (Was it grass pollen or leaf mediated? Was it mould mediated? Was it even IgE mediated?)
- while the dogs were both significantly improved, especially in ventral skin regions, they were still on low levels of prednisolone, albeit much reduced compared with initially required doses.

There leaves a few possibilities that may explain the clinical observations.

Induction of tolerance to relevant grass contact allergen(s)

There are two broad possibilities as to how ASIT could induce tolerance to grass contact allergens. Firstly there may have been crossreactivity between related grass pollen and leaf proteins. The most common turf grasses in Melbourne are either Stenotaphrum spp (Buffalo grass) or Pennisetum clandestinum (kikuyu) and these are both members of the Panicoideae subfamily, Panocodae group and Paniceae subtribe of grasses along with Paspalum notatum. While the former two grasses are not available as commercial pollen allergens Paspalum, is and was in the vaccine for both these dogs.

A second possibility would be more extensive panallergens. Both cases showed extensive crossreactivity to grasses and other pollens. In humans, it is considered likely that reactions to pollen originating from multiple sources are frequently due to sensitisation to highly conserved allergens (panallergens) involved in vital plant processes rather than to genuine sensitisation due to exposure to pollen from various species, though the clinical significance of panallergens remains controversial. In dogs, the existence of panallergens has not been proven but Western blot data presented by Mason and Ruutu support this possibility. Interestingly in case one, all tree pollens bar cypress were positive and in humans, profilin panallergen coreactivity with cypress has been noted to be rare. In these cases, if there existed a relevant panallergen present in both contact allergy-inducing leaf as well as vaccine pollen extracts then desensitisation should be possible.

Both these scenarios would potentially explain the clinical presentation, the test results and the response to ASIT.

PAR2 mediated improvement

Proteinase activated receptor-2 (PAR2) plays a significant role in human itch, and likely also in dogs. Both endogenous and exogenous proteases (including dust mites & cockroach allergens and Staphylococcus aureus exotoxins) can trigger PAR2 on keratinocytes leading to TSLP (thymic stromal lymphopoietin) mediated itch. Plant and mould allergens can act as serine proteases potentially triggering PAR2 mediated itch.

In these cases it would need to at least be considered that reduction in PAR2 expression with successful ASIT could improve clinical signs associated with direct triggering of PAR2 by contact with grass and/or grass-associated mould allergens.
Other possibilities
Less likely possibilities to explain the clinical observations in these cases include

• improvement in barrier function with ASIT leading to reduction in percutaneous absorption of allergens may explain improvement in ‘contact’ allergy clinical signs
• induction of tolerance to conventional pollens (if they were responsible for the presenting signs) and subsequent clinical improvement with ASIT.

However, both these possibilities raise a further issue in what makes these dogs different from ‘classic’ atopic pollen allergic dogs where avoidance of grass does not result in improvement of clinical signs?

Conclusion
Despite questions over the mechanisms present in these cases, two conclusions can be made:

• Grass avoidance can be of significant help in some dogs with demonstrable IgE to multiple grass pollens and clinical signs consistent with contact allergy concurrent with more conventional signs of atopic dermatitis.
• ASIT can assist with control of clinical signs consistent with contact dermatitis in at least some dogs with concurrent atopic dermatitis and contact dermatitis and demonstrable IgE to multiple grass pollens.

References
2. Mason KV & Ruutu M. New allergens in canine atopic disease – or is it contact atopy? Australian and New Zealand College of Veterinary Scientists Dermatology Chapter Science Week Proceedings, Gold Coast, 12-13th July 2013: 51-54 (accessible via free download at http://dermatology.anzcvs.org.au/dermatology/publications/proceedings_2013/; note though that Western blot data was not included in the proceedings).


**Update on the antimicrobial susceptibility of clinical isolates of**

*Staphylococcus* in Australian dogs and cats

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**Antimicrobial susceptibility testing - clinical breakpoints vs epidemiological cutoffs (AKA microbial breakpoints or ‘wild-type’ breakpoints) – what does it all mean?**

As every clinician knows, the purpose of submitting a sample for cytology/histopathology, microbial culture and antimicrobial susceptibility testing (AST) is to determine the cause of the disease and the options available for effective therapy. Clinical microbiological labs often communicate the results of AST as simply ‘susceptible’, ‘resistant’ or on some occasions ‘intermediate’. This leaves us with the impression that AST determination is a simple binary or tertiary category set in stone. All AST require the prior establishment of ‘breakpoints’ (aka interpretative criteria) and it is here where things get complicated.

The term ‘breakpoint’ is variably used. One use of the term ‘breakpoint’ is microbial focused ie it centers on what is happening in that population/genera/species of bacteria. In this context it is used to describe a **‘wild-type breakpoint’** (AKA ‘microbial breakpoint’) which is the minimum inhibitory concentration (MIC) of an antimicrobial agent that distinguishes the wild-type population of bacteria (those without acquired and mutational resistance mechanism to a drug) from the non-wild type bacteria (those with acquired or mutational mechanisms). To remove confusion, the preferred term suggested now by the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org) is **‘epidemiological cut-off’ (ECOFF) value**. To create these values for each antimicrobial agent requires data from a large number of in vitro MIC tests on each bacterial species/genera. While it is microbial focused, the information to determine the ECOFF is derived from large bacterial populations so has great value in determining ‘clinical breakpoints’ (described below) and in monitoring changes in antimicrobial resistance.

**Clinical antimicrobial susceptibility breakpoints** are used to predict the clinical outcome of antimicrobial treatment. It refers to the concentrations of an antibiotic (MICs) that ‘separates between strains where there is a high likelihood of treatment success from those bacteria where treatment is more likely to fail’. These breakpoints are often derived from prospective clinical studies comparing outcomes with the MICs of the infecting pathogen.

Finally **‘pharmacokinetic/pharmacodynamic (PK/PD) breakpoints’** is the antibiotic concentration derived from a practical understanding of what the body does with the drug (pharmacokinetics) and what the drug does to the microorganism when it’s in the body (pharmacodynamics).

Therefore the so called simple request each clinician makes when submitting a clinical sample (‘what antibiotic is useful in treating this infectious disease’) really needs consideration of all three of the above ‘breakpoints’ ie the wild-type distribution of MICs and ECOFF value; an understanding of what the body does with the drug and what the drug does to the bacteria when in the body (PK/PD breakpoint) and knowledge of the clinical outcome of infections when the antibacterial is used which takes into account all three of the above ‘breakpoint’ types. These things may change when antibacterial agents have been in clinical use for some time, and so need to be reviewed frequently especially as new mechanisms of resistance are discovered and in some cases multiple mechanism of resistance may be available to a bacterium against a given antibiotic. This makes the job of a veterinarian in treating infectious disease more complex than human medical practitioners, given the variability in the animal species we treat (with potentially variable PK/PD) and especially in the case of *Staphylococcus sp*, the bacterial species variation hosted by each animal species.
Figure 1: Determination of epidemiological breakpoints of susceptibility to antibiotics. Epidemiological breakpoints are determined from the analysis of minimum inhibitory concentrations (MICs) of a large number of isolates. The number (or proportion) of isolates that have particular MICs is plotted to obtain a distribution curve. The common epidemiological cut-off value for resistance (ECOFF) breakpoint is established as the upper limit of the curve for the majority of the population. Those isolates that have MICs above this breakpoint are considered resistant to the antibiotic even if their MICs do not reach the clinical breakpoint, which predicts clinical success. Breakpoints are usually defined using double dilution methods, which means that the explored concentration range has a geometric distribution that can hide small changes in MIC. If the MICs of the majority of the population, as obtained using double dilution tests, are broken down using arithmetic antibiotic concentrations to establish MICs (see inset graph), subpopulations that have subtle differences in their MICs can be detected, and new (usually lower) ecological breakpoints that define the most susceptible population can be established. The double dilution method makes it difficult to detect populations with low-level antibiotic resistance that can eventually evolve into populations with high-level resistance\(^6\), which is an aspect that has been overlooked in the past 50 years of routine application of susceptibility testing methods, and which might be the basis of the "MIC-creep" that is observed for different antibiotics\(^5\).

Figure 2: Ciprofloxacin MIC distribution of *Escherichia coli* isolates (http://www.eucast.org). Epidemiological cut-off (ECOFF) values and clinical susceptible (S) and resistant (R) breakpoints from CLSI and EUCAST committees are indicated. The clinically susceptible population (below the clinical susceptible breakpoint) includes part of the microbiologically resistant population (low-level resistant bacteria, presumably expressing *qnr*-like genes or other PMQR mechanisms or first step *gyrA* mutations) and the wild-type population (below the ECOFF value and presumably without resistance mechanisms). The clinically resistant population (beyond the clinical resistant breakpoint) includes isolates with high-level resistance mechanisms (most probably double-step *gyrA* mutants or a combination of *gyrA* with *parC* mutations) Canton et al 2011. Microbiology reviews.
Methicillin resistance (MR) vs Multidrug resistance (MDR) vs Extensive drug resistance (XDR)
The emphasis of many research projects and clinical based papers in Staphylococcus species in veterinary and human medicine has been on the presence or absence of ‘methicillin resistance’. The term ‘methicillin resistance’ is an historical misnomer inferring resistance to a single penicillin antibiotic (methicillin) but in reality referring to the presence of ‘pan-beta-lactam resistance’. This ‘pan-beta-lactam’ resistance results from a structural change in penicillin binding proteins in the bacterial cell wall that stops the binding of the beta-lactam ring (present in all penicillins, cephalosporins, carbapenems, monobactams and penems), thereby making the drug unable to interrupt bacterial cell wall construction and stabilization, which is the usual way beta-lactam antibiotics kill the bacteria (bacterial cell lysis). 'Methicillin resistance' is commonly mediated by the meca gene which codes for the supplemental penicillin binding protein 2A (altered transpeptidase) and is expressed homogenously (all bacteria) or heterogeneously (more difficult to detect phenotypically as only a fraction of the bacterial population express it so care is needed in antimicrobial susceptibility testing). With many of the beta-lactam antibiotics having been the mainstay of antimicrobial therapy for clinical infections involving Staphylococcus species, the presence of resistance to these drugs has been a reasonable concern.

An even greater concern with respect to antimicrobial resistance is in the emergence of multi-drug resistant (MDR) or extensively drug resistant (XDR) bacteria in our veterinary clinical isolates, especially in Staphylococcus pseudintermedius. There is controversy over the definition but a recent classification by Magiorakos and colleagues (2012) defines MDR as non-susceptible to at least one agent in 3 or more categories of antimicrobial agents while XDR is non-susceptibility to at least one agent in all but two or fewer antimicrobial categories i.e. bacterial isolates remain susceptible to only one or two categories.²

What staphylococcal species are occurring in Australian dogs and cats, is antimicrobial resistance common, where are the origins of the resistant strains? The story so far
At a national level, a one year survey (Jan 2013-Jan 2014) involving 22 veterinary diagnostic labs in Australia was the first nation-wide survey of antimicrobial resistance of clinical isolates of Staphylococcus and E.coli isolates in animals. This 12 month survey was kindly funded by Zoetis and leaders of this amazing collection and storage of isolates were Dr Sam Abraham and A/Prof Darren Trott. The broader research study which then included analysis of the isolates collected were then used in the ARC linkage funding (LP130100736: ‘Superbugs and veterinary drugs: Are multidrug-resistant zoonotic pathogens residing in Australian animals?’ Trott, Schembri, Norris, Gibson, Beatson), with industry partners Zoetis (Pfizer Australia), Luoda Parma, Biosecurity Queensland, NSW DPI and WA Dept of Ag and food.

Analysis to date has confirmed a total of 1199 clinical isolates to be Staphylococcus isolates. Epidemiological data including animal species, sample site and clinical syndrome were recorded along with microbiological data. Of these 811 have been derived from dogs (n=737; 91%) and cats (n=74; 9%). The isolates have undergone species confirmation via a range of phenotypic and molecular testing (including MALDTI-TOF). S. pseudintermedius was the most commonly isolated species in dogs (82%) followed by S.aureus and S.schleiferi. In cats, S.felis (50%) was the most common followed by S.aureus and S.pseudintermedius. The antimicrobial resistance profile of each isolate was determined by disc diffusion and minimum inhibitory concentration in accordance with Clinical Laboratory Standards Institute (CLSI). Isolates found to demonstrate methicillin resistance as determined by phenotypic resistance to oxacillin using established breakpoints were further characterised using whole genome sequencing to enable typing as well as detection of the presence/absence of known antimicrobial resistance genes to each of the antimicrobial classes.

Antimicrobial resistance including methicillin resistance was notably absent from most S.schleiferi in dogs (ears; 43/44) and in the single MR isolate, susceptibility to all other antimicrobial agents was found. S. felis did not demonstrate any isolates with MR, MDR or XDR. Methicillin resistance was present in 10% of canine isolates (73/737) and 11% of feline isolates (8/74). All Staphylococcus species had at least one MRS except for the S.felis as noted. Genome sequencing found that ST71, the dominant strain of methicillin-resistant S. pseudintermedius (MRSP) in Europe, is also a common strain in Australia. We identified several new strains of MRSP that will be further characterised. One of these strains, found only in dogs on the east coast, was resistant to all antimicrobials except rifampicin. Such extensive drug resistance was not seen in feline samples and in the two MRSP isolated from cats, both appeared to be new sequence types. Most of the methicillin-resistant S. aureus strains (15/16) were of human origin (ST8, ST22).

This work on antimicrobial susceptibility testing, Staphylococcus speciation and typing via whole genome sequencing has been performed by PhD candidates Kate Worthing (USYD) and Sugiyono Saputra (Uni of Adelaide). Further analysis of this work and the significance of the findings of WGS and AST are currently been undertaken which will be discussed during the presentation.
MRSP ST71 – the sticky bug?

ST71 is the most common MRSP clone in Europe in dogs and cats, and has also been associated with infections in humans. First detected in Europe in 2007 it has spread within this continent as well as in countries outside Europe such as Brazil, USA and Canada and now is evident in Australia. The multidrug resistance patterns displayed by some MRSP lineages and typically displayed by this sequence type (ST) has been concerning. Latronico and colleagues (2014) evaluated the adherence properties of MRSP and MSSP, showing that MRSP ST71 adhered better to canine and human corneocytes than non-ST71-MRSP and MSSP which they concluded may explain the epidemiological success of this clone. MRSP ST71 has recently been shown to have a greater ability to produce biofilm compared with other STs. Genetic factors associated with these phenotypic traits remain to be identified but it is likely that this clone’s particular ability to adhere to skin and form biofilm more so than other members of the species are important virulence factors.

References

The incidence of canine methicillin-resistant *Staphylococcus pseudintermedius* pyoderma in a specialist dermatology practice over a 3 month period in 2014: a prospective study

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Introduction

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

MecA-confirmed MRSP was first reported in 1999 in the US. Since then, MRSP has been reported from Europe, Asia and the middle East. It was first reported in Australia by Siak et al with cases from 2011. Canine MRSP pyoderma was first diagnosed at the referral practice of the author (ASES) in May 2013.

There have been multiple reports of the prevalence rates of MRSP in dogs and cats globally, though the majority of these have been either prospective surveys of carriage in healthy patients and/or hospital patients, or retrospective studies based on laboratory data. A single study has examined the prevalence of MRSP specifically in dogs with pyoderma. That Japanese study reported that 66% of the S. pseudintermedius isolates cultured from dogs with pyoderma visiting two referral hospitals were methicillin-resistant based on the detection of the mecA gene.

The primary aim of this study was to define incidence of MRSP in dogs with pyoderma presenting to the Animal Skin and Ear Specialists (ASES) over a three month period in late 2014. An additional aim was to look for any variation in incidence and patterns of antibiotic sensitivity between cases presenting to the Essendon (ESS) branch practice (located in the inner north west of Melbourne) and the Glen Waverley (GW) clinic (located in the middle south east of Melbourne).

Methods

Cases were presented to the ASES from September 19 to December 19 2014. Inclusion criteria included any dog with a bacterial pyoderma (surface, superficial or deep) with consistent cytological findings (neutrophils with intracellular cocci) determined by the clinician to require oral antibiotic therapy. At least one follow up revisit to determine success or failure of therapy was mandatory, otherwise a case was considered lost to follow up (LTFU). If empirical therapy failed then a culture and sensitivity was performed. Culture techniques varied depending on the case and included dry swabs, sterile saline swabs, pustule exudate sampling and biopsy and tissue culture. All samples were submitted to the same commercial laboratory and underwent testing according to CLSI (Clinical and Laboratory Standards Institute) methodology. Cases were thus classified as responders to empirical therapy (RTET), MRSP or LTFU.

The confirmed incidence of MRSP pyoderma at each geographic site (ESS and GW) as well as the total overall was then calculated as the number of confirmed MRSP cases divided by the total number of included cases. The possible upper range of the incidence was calculated by adding MRSP and LTFU cases and dividing by the total number of cases.

To assess overall antibiotic resistance trends between antibiotics, for each antibiotic the number of sensitive isolates was totalled. To assess any antibiotics resistance trends between the different geographic locations, for each antibiotic and each location the number of resistant isolates was expressed as a percentage of the number of cases seen at the site. The results between practice locations were tested using Fisher’s two tailed test for significance.

Lastly, the patterns of sensitivity and resistance for each MRSP isolate were analysed and each distinct pattern (assuming intermediate results were resistant, and ignoring doxycycline results because of inaccuracy compared with...
other MRSP isolates) to assess whether any particular antibiograms (and thus possible MRSP strains) were more frequent than others.

**Results**

95 cases were included in the study (GW 70, ESS 25). The confirmed incidence of MRSP was 17.9% (GW 18.6%, ESS 16.0%) with a maximum possible incidence of 31.6% (GW 31.4%, ESS 32.0%) once LTFU cases were taken into account.

Multiple antibiotic resistances were common in MRSP isolates with <25% MRSP isolates sensitive to tetracycline, erythromycin, clindamycin, lincomycin, and enrofloxacin. Only fusidic acid, rifampicin and vancomycin were reported sensitive in >90% isolates tested. When comparing results between GW and ESS, isolates from ESS appeared to show less resistance compared with GW for tetracycline, erythromycin, clindamycin, lincomycin and chloramphenicol but these differences were not significant.

Ten different antibiograms were found from the 17 isolates. At the ESS location no repetition was seen in antibiograms, and at GW one antibiogram was isolated three times, one antibiogram two times, and the remainder one time each.

**Discussion**

MRSP is a common organism in the caseload seen at ASES with the organism isolated in at least 16% of canine pyoderma cases requiring systemic antibiotics. The incidence was similar between both GW and ESS. It should be noted that because of the design of this study, this incidence should not be taken as an estimate of the prevalence of MRSP in the general population of dogs in Melbourne which is likely very much lower than the findings of this study. It should also not influence selection of initial empirical therapy to canine pyoderma, especially first presentations. Larsen et al suggests that the prevalence of some drug resistance in canine pyoderma is less in cases presenting for the first time compared with revisit cases, and the majority of cases in this study were diagnosed on revisits rather than initial consultations (data not shown).

Resistance to multiple antibiotics was common in MRSP strains in this study. This emphasises the need to culture to identify susceptible antibiotics where MRSP is suspected. The author and the other dermatologists at ASES will typically use two concurrent susceptible-reported antibiotics for the treatment of MRSP to minimise the risk of selection of further antibiotic resistance.

Despite being a crude tool for this purpose, the identification of multiple antibiograms suggests multiple MRSP strains prevalent in the Melbourne region. This variety of antibiograms also suggests, that while veterinary hospitals are reported to represent a significant risk factor MRSP infection, acquisition of infection directly or indirectly at ASES does not appear to be the primary source in most of these cases.

There were several limitations in the study that may have lead to inaccuracies in the results. Limitations that may have lead to underestimation of the MRSP incidence included

- the number of LTFU cases
- MRSP cases responding to empirical use of clindamycin, marbofloxacin, enrofloxacin or doxycycline
- MRSP cases responding to topical therapies used in conjunction with empirical therapies
- reliance on sensitivity testing for identification of MRSP cases (some MRSP cases are still missed by this testing alone; meCA PCR detection remains the gold standard)

Limitations that may have lead to overestimation of the MRSP incidence included

- several empirically responding clinically diagnosed cases removed from the study because of lack of definitive cytology (data not shown)
- cases removed from the study with focal lesions just treated topically

Larger case numbers may also have clarified any differences in antibiotic resistance trends between the two locations.

**Conclusion**

MRSP is clearly present in Melbourne. Practitioners need to be aware of this and be prepared to culture cases that are not responding to appropriate empirical antibiotic therapy. Practitioners need to have hospital protocols ready to
minimise the risk of direct and indirect spread of MRSP between cases. Repeating this study in future years should detect trends in antibiotic resistance in cases of MRSP canine pyoderma in Melbourne.

A. Gribbles Pathology (Vic) Pty Ltd, Clayton VIC

References


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In-hospital management of methicillin-resistant Staphylococcus: the Melbourne Veterinary Specialist Centre experience

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Introduction

The risk of methicillin-resistant Staphylococcus pseudintermedius (MRSP) colonisation and infection in dogs has been reported associated with medication use (recent (30 days) antibiotic use, antibiotic use in the last 12 months, topical ear medication, glucocorticoid use), veterinary hospitals (hospitalisation, frequent (>10) visits), and higher dog density in cities.1-3 Within the hospital environment, isolation of MRSP has been reported post disinfection, and in a longitudinal study isolation of MRSP was variable over time and this suggests that environmental decolonisation of MRSP can be difficult.1,4 Veterinary staff, their clothes and their mobile phones may also be colonised with MRSP.5-10 Given the significant risk veterinary hospitals represent to veterinary patients for acquisition of MRSP, it is imperative that in-hospital protocols be formulated and implemented to keep this risk to a minimum. Broad guidelines have already been published for management of MRS1,11-14 and these references should be used by the reader for design of in-hospital infection management, but practical implementation of these from hospital to hospital will differ.

Following the identification of the first case of MRSP in May 2013 at Melbourne Veterinary Specialist Centre, it became apparent there was an urgent need for better protocols, documentation and education of both staff and clients to minimise the risk of MRSP spread between humans, animals and the hospital itself. What follows is a summary of this experience and the written protocols. Appropriate management of the MRSP patient and appropriate antibiotic use is beyond the scope of this presentation.

Identification of MRSP

The first step in hospital management of MRSP is identification of affected cases. Culture is warranted in cases of cytologically confirmed coccal pyoderma where there is failure of response to appropriately dosed β-lactam antibiotics (including clavulanic acid-amoxicillin, cepalexin and cefovecin) or two different non-β-lactam antibiotics (e.g. enrofloxacin, clindamycin). Failure of response typically consists of new infection lesions (confirmed on cytology) or persistence of all or some of the initially identified lesions. New or persistent lesions may only be mild – lack of severity of the lesions should not influence the decision to culture. Appropriate culture techniques may include swabs from intact pustules, dry swabs from under crusts, moist swabs from epidermal collarettes or tissue cultures depending on the decision of the clinician.15 While a culture is pending the record is marked with a ‘(%)’ after the patient name to indicate the case may be an MRSP carrier. Once a case has been identified as MRSP positive, the brackets are removed and the record is marked with a ‘%’ after the patient name, as are all other animals in the household (as concurrent carriage is assumed until proven otherwise).

Following successful treatment of any MRSP infections, if there is no relapse of infection for a 5-6 month period then repeat culture for carriage is recommended. This is typically taken from the oral cavity, then nostril, then perianal region on the one swab, and the lab requested to assess for Staphylococcus specifically in what it likely a mixed culture. If this culture is negative, then the other animals in the household are cultured similarly and if all are negative then the patient (and home environment) is assumed decolonised and the files are marked with ‘#’ to indicate this status change.

Culture is recommended strongly in any subsequent breakout infection lesions in cases marked with ‘%’ or ‘#’ on the file.

Education

A lack of understanding of MRSP by both staff and owners can rapidly lead to fear of the unknown, and easy confusion with methicillin-resistant Staphylococcus aureus, which represents a far more significant risk to human health. The take home message for MRSP is simple – it is no more contagious or disease causing to animals or humans than the more sensitive strains of S. pseudintermedius they have been exposed to for years. Its’ one and only advantage is survivability in the face of antibiotic therapy. Many animals (and fewer humans in contact with those humans) carry MRSP without clinical disease. However, MRSP is important in animals who are prone to bacterial
infections, typically (though not confined to) allergic dermatological patients and post surgical patients. Restricting spread of MRSP to these patients in particular is the goal of MRSP hospital hygiene.

Staff
All staff need to be aware of MRSP management protocols. Reception and kennel staff in particular represent a challenge as they a critical part of management but may lack scientific training. Internal documentation should include a general MRSP information handout, written protocols for handling of MRSP carrying outpatients and inpatients, and written protocols for veterinarians regarding diagnostic procedures, marking of records and dissemination of information. Development of this documentation should involve all the affected parties to maximise practicality and long term compliance.

Once MRSP was identified at MVSC these handouts and protocols were developed and revised several times for the staff. In the early days, ongoing modifications to the various protocols for handling of MRSP patients, as problems were identified and solved, posed an educational challenge as changes were not infrequent.

Clients
Owners of animals with MRSP are a critical component of in-hospital MRSP management. There are three important phases of an MRSP infection that the owners need to be aware of – initial diagnosis, infection clear but still a carrier, and decolonised. Handouts should be developed for each of these phases of treatment which specifically detail MRSP biology, the client role in MRSP management, what to do and what to expect at subsequent hospital visits.

Other pet owners also need to be aware of MRSP as they may see some of the hospital management protocols and fear the worst with respect to contagion to their own pets. Educational signs and handouts, and effective education of reception staff to reassure these clients is important.

Other Veterinarians
In referral practice, all animals come from other veterinary clinics. Once a patient has been diagnosed with MRSP the referring veterinarian needs to be made aware of this if the MRSP diagnosis has not been previously made because this has implications for management of that hospital when the MRSP patient returns to the referring vet. An informational should be developed for this purpose.

Hand Disinfection and Glove Use
Routine hand disinfection remains the most important activity in the control of nosocomial infections (including MRS) in practice. As per WHO guidelines, hands should be washed with soap and water when visibly dirty or visibly soiled with blood or other body fluids or after using the toilet. Use of an alcohol-based handrub is the preferred means for routine hand antisepsis in all other clinical situations, if hands are not visibly soiled. Soap and alcohol-based handrubs should not be used concomitantly.

At MVSC 3M Avagard alcohol based hand rub dispensers are located at the entrance and exit of consult rooms, the main building, kennels and in the procedure room. 3M Avagard liquid soap dispensers are used at sinks.

Hand hygiene should be performed immediately before and after patient contact (and prior to contact with equipment including keyboards and microscopes), before handling an invasive device for patient care, after contact with body fluids, excretions, mucous membranes, non intact skin or wound dressings, after removing gloves, and before handling medication or preparing food.

Disposable gloves should be used for any contact with known or suspect MRS colonised animals, and when it can be reasonably anticipated that contact with potentially infectious material, mucous membranes, or non intact skin will occur.

Ideally no jewellery (rings, bracelets), wristwatches, nail polish or fake nails should be worn and nails should be kept short and clean.

Overzealous use of alcohol rubs or soaps can lead to dermatitis of the hands and thus a higher risk of MRSP/MRSA carriage. Moisturising regularly should prevent this. Moisturiser is provided adjacent to handwashing points.
Note that staff with major skin barrier defects (e.g. eczema, psoriasis, open wounds etc.) or who are immunosuppressed should not nurse MRS positive animals. Where this is a possible concern medical health advice should be sought.

Routine floor and room disinfection

70-90% alcohol spray (kills Staphylococcus in 10 seconds)\textsuperscript{17} is used to clean the examination table between clients, and benchtop and keyboards daily in all consultation rooms. The floors are cleaned with detergent if soiled, followed by 1:250 F10 (kills Staphylococcus in between 15 seconds to <5 minutes).

\textbf{F10} (as opposed to F10SCXD) is a disinfectant not a floor cleaner. It can be inactivated by organic debris. If there is gross contamination of the floor (ie dirt, faeces) then cleaning PRIOR to the use of the F10 with a detergent floor cleaner only, then dry mop, then F10 is strongly recommended. Hair does need to be removed as this can harbor MRSP. The vacuum cleaner can be used but the head would then need to be cleaned and treated with f10 to prevent spread by the vacuum cleaner.

Staff Clothing
Veterinary staff clothing is recommended to be hot washed daily at a temperature of >60°C.

Specific MRSP Protocols

A strict protocol is used for booking confirmed MRSP carrying patients. This is based around the possibility that some of the lateral transmission of MRS that occurs between patients occurs though direct or indirect contact in the waiting room and procedure rooms.\textsuperscript{12} To this end cohorting of the risk group (in this case division of known MRS carriers from the rest of the animal population) is aimed to be achieved.

\textbf{At MVSC most of the following is the responsibility of the departmental nurses except where otherwise noted.}

Consultations

Booking

MRSP visits can only be booked with half hour gaps between them to allow for cleaning and disinfection times. As MRSP strains can vary in resistance, cleaning between cases ensures no cross contamination between dogs carrying different strains of MRSP. A separate MRSP room schedule is maintained on the booking system to avoid double booking the MRSP room.

\textbf{MVSC has a consult room that is only used for MRSP cases. The keyboard in the room has flat keys that are easy to disinfect.}

Before the consultation

Extendible ‘airport’ barriers are placed in the waiting room running from the entrance of the MRSP isolation consult room to near the main entrance. Laminated warning signs are hung from the barrier to keep other patients away from the affected region. The MRSP mop & bucket is filled with F10 diluted 1:250 with water. An appropriately sized MRSP PPE (personal protection equipment) kit is retrieved for the vet (contains shoe covers, disposable gown, appropriately sized gloves, and disposable scrub hat & mask if required). The client and pet can wait in car or in the barriered waiting room area prior to the consult.

The consultation

When ready for consult the client ideally carries small dogs straight in to the room from the car, or larger dogs are walked in via the barriered region. Once the client enters the building with the dog, the door handles to the building should be cleaned with alcohol wipes. Reception staff are responsible for this.

The main door entrance to the waiting room, through to the barrier area should be mopped with 1:250 diluted F10 followed by the wet floor sign. After 2 minutes this can be dry mopped. As the client goes into the MRSP room with sign on the door should be changed to MRSP not clean. The veterinarian changes into the provided PPE and performs the consultation.

After the consultation (reception)

The vet disrobes in the MRSP room and steps out. The client is left with the pet in the MRSP room till released by the nurse. Clients are then encouraged to put the dog directly in the car. Client should be encouraged to use alcohol gel once at reception to minimise contamination of pens / pinpads / reception bench etc. Once invoice is settled if the pet is
not already in the car then pets are transported to the car. Once the client has left the reception top, pinpad and door handles are alcohol wiped. The barriered region and the main door entrance are mopped with 1:250 F10 and water and the wet floor warning sign is put out. After 2 minutes the floors may be dry mopped and the barriers removed.

After the consultation (MRSP room)
Everything above the floor that was potentially contaminated is cleaned with either 70-90% alcohol. This includes the examination table, chairs, keyboard, and door handles. It is best to spray paper towel then wipe clean (or spray then wipe with paper towel) to get even coverage. The floor is mopped with 1:250 F10 and water. After 2 minutes the floors may be dry mopped.

End of Day
Veterinary PPE bags are restocked and the MRSP mop head is hot washed.

Several other disinfectants were trialled before settling on the F10. Various problems encountered included stripping the Tarkett flooring, slipperiness on the floor after drying, stickiness on the floor after drying, and buildup of detergent on the floor with repetitive usage. Several had 5-10 minute disinfection times also which was impractical.

Hospitalisation
Known MRSP cases that required hospital procedures represent a challenge for management. Cohorting the MRSP cases is still targeted by having the cases as the last admission, last procedure and first discharge to try and aim for the shortest in hospital time. The patients are caged in the MRSP room in a collapsible cage until the time of the procedure with admission and discharge procedures similar to MRSP consultations. At the time of the procedure all staff wear PPE, and the areas of the treatment room being used is barriered off to avoid contact with other staff and pets. Following the procedure equipment and tabletops are disinfected with 70-90% alcohol, and the floors again mopped with 1:250 F10. The floors between the MRSP room and the procedure room are mopped immediately following transport of the patient to and from the MRSP room.

The Future
In an ideal situation a proactive monitoring program of environmental culture would be implemented to assess success of the protocol including sampling of both the environment and veterinary personnel (hands, clothes, shoes, phones) every 4 months. Additional information on and problem ‘hospital strains’ of MRS would be gained by typing (pulse field gel electrophoresis and spa typing) of any MRS bacteria isolated.

However, given the variability in outcomes when this has been done recommendations against it and ongoing cost the usefulness of this is debatable. What is more practical is ongoing reviews of practicality and implementation of the existing protocols.

Conclusion
Development of successful in-hospital management protocols for MRSP need to involve all staff as well as the clients. It is a significant task that will likely need frequent modifications (and patience) especially in its early stages. Once an effective MRSP hospital plan is in place, it is beneficial for both staff and clients (because of better education and well defined roles leading to a lack of uncertainty) and patients (because of reduced risk of MRSP contagion).

If you wish for a copy of any of the protocols mentioned here they may be downloaded from the MVSC website at http://www.melbvet.com.au/ in the dermatology information section (application required to access).

References


In-vitro sensitivity of methicillin-resistant Staphylococcus pseudintermedius to tetracycline and doxycycline by CLSI methodology

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Introduction

Methicillin-resistant Staphylococcus pseudintermedius (MRSP) canine pyoderma is a growing problem in Australia. One of the primary challenges associated with treatment of this often multi-drug resistant organism is finding effective systemic antibiotic therapy. In Australia, bacterial culture and sensitivity tests performed using disk diffusion techniques are most commonly used to achieve this.

The problem for the author initially arose when it was noted there was an inordinate number of results which were reported resistant to tetracycline but sensitive to doxycycline, and whether doxycycline would be an effective antibiotic in these cases. The 2013 Clinical Laboratory Standards Institute (CLSI) standards for susceptibility testing of bacteria of veterinary interest did not clarify this, stating strains susceptible to tetracycline should be regarded as susceptible to doxycycline, whereas strains that have intermediate resistance or are resistant to tetracycline may be susceptible to doxycycline. This contrasts with reports in humans with methicillin-resistant Staphylococcus aureus infections where tetracycline resistance predicted doxycycline resistance in all cases, and in most animals, where tetracycline resistance predicted doxycycline resistance in 97% of MRSP isolates.

A case of MRSP pyoderma in a dog managed by the author which was clinically nonresponsive to appropriate doses of doxycycline but was repeatedly reported on testing as sensitive doxycycline but resistant to tetracycline prompted further investigation of the problem.

Many different tetracycline resistance genes are present in bacteria, but the tetracycline resistance genes reported present in MRSP (table 1) did not offer a possible explanation for the observed issues.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efflux Pumps: code for membrane-associated proteins which export tetracycline from the cell, reducing the intracellular drug concentration and thus protects the ribosomes within the cell. Most efflux proteins confer resistance to tetracycline and doxycycline but not to minocycline.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TetK</td>
<td>Plasmid</td>
<td>More common in ST71 clone (‘European’)</td>
</tr>
<tr>
<td>TetL</td>
<td>Plasmid</td>
<td>Rarely reported</td>
</tr>
<tr>
<td>Ribosomal Protection Proteins: code for cytoplasmic proteins that protect the ribosomes by binding to them leading to allosteric disruption of tetracycline binding site. They confer resistance to all tetracyclines.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TetM</td>
<td>Chromosome or transposon</td>
<td>More common in ST68 clone (‘North American’)</td>
</tr>
<tr>
<td>TetO</td>
<td>Chromosome or transposon</td>
<td>Rarely reported</td>
</tr>
</tbody>
</table>

A recent publication by Maaland et al (2013) offered another possible explanation. Because the current CLSI breakpoints for Staphylococcus in the dog are based on human clinical breakpoints, the authors of that paper set out to (i) determine canine-specific interpretive criteria for establishing breakpoints for doxycycline and (ii) to evaluate the use of tetracycline as a surrogate drug for interpretation of the doxycycline susceptibility of canine S. pseudintermedius isolates. Following pharmacokinetic / pharmacodynamic analysis, the paper confirmed the human breakpoints were inappropriate for canine S. pseudintermedius isolates, and proposed new breakpoints for MIC and disk diffusion testing which were significantly lower than the human breakpoints.

The hypothesis of this study was that when these proposed breakpoints were applied to discordant tetracycline resistant / doxycycline sensitive results in MRSP cases, that the new interpretation would support doxycycline resistance.
Materials and Methods

All isolates of MRSP cultured from canine pyoderma from cases visiting Animal Skin and Ear Specialists at Melbourne Veterinary Specialist Centre between 18th September 2014 and 3rd February 2015 for which tetracycline and doxycycline zone of inhibition (ZoI) data were entered into the study. Reported CLSI interpretations\(^5\) of disk diffusion testing was recorded, and ZoI measurements were reinterpreted as per Maaland et al (2013) (Table 2) and these results recorded.

| Table 2: Breakpoints for interpretation of tetracycline and doxycycline sensitivity of canine \textit{S. pseudintermedius} isolates using disc diffusion testing |
|---|---|---|
| susceptible | ≥19 | ≥23 |
| intermediate | 15-18 | 18-22 |
| resistant | ≤14 | ≤17 |
| **Doxycycline** | | |
| susceptible | ≥16 | ≥25 |
| intermediate | 13-15 | 21-24 |
| resistant | ≤12 | ≤20 |

* Breakpoints for tetracycline as per Maaland et al (2013) are as surrogates for doxycycline.

Results

17 isolates satisfied entry criteria (table 3). Based on CLSI interpretation\(^6\) 6/17 isolates were sensitive to both tetracycline and doxycycline, 2/17 isolates were resistant to both tetracycline and doxycycline, 7/17 isolates were resistant to tetracycline and sensitive to doxycycline, and 2/17 isolates were resistant to tetracycline and showed intermediate sensitivity to doxycycline.

Of 9/17 discordant results, all nine, when interpreted using the proposed breakpoints of Maaland et al (2013), were found to be resistant to doxycycline.

| Table 3: ZoI and interpretation of 18 isolates of canine MRSP sensitivity to tetracycline and doxycycline |
|---|---|---|---|---|
| **Case** | **Zone of Inhibition (mm)** | **CLSI Interpretation** | **Proposed Interpretation** |
| | TET | DOXY | TET | DOXY | TET | DOXY |
| 1 | 23 | 30 | S | S | S | S |
| 2 | 30 | 32 | S | S | S | S |
| 3 | 27 | 30 | S | S | S | S |
| 4 | 30 | 31 | S | S | S | S |
| 5 | 33 | 35 | S | S | S | S |
| 6 | 28 | 30 | S | S | S | S |
| 7 | 8 | 14 | R | I | R | R |
| 8 | 9 | 15 | R | I | R | R |
| 9 | 10 | 17 | R | S | R | R |
| 10 | 10 | 20 | R | S | R | R |
| 11 | 10 | 16 | R | S | R | R |
| 12 | 9 | 16 | R | S | R | R |
| 13 | 9 | 16 | R | S | R | R |
| 14 | 9 | 16 | R | S | R | R |
| 15 | 10 | 18 | R | S | R | R |
| 16 | 0 | 0 | R | R | R | R |
| 17 | 0 | 0 | R | R | R | R |

Key: TET=tetracycline, DOXY = doxycycline, R=resistant, S=Sensitive, I=intermediate; * Breakpoints for tetracycline as per Maaland et al (2013) are as surrogates for doxycycline.

Discussion

Based these data and the proposed interpretative breakpoints of Maaland et al (2013)\(^1\) it would appear that, when CLSI testing methods using current recommendations are used \textit{in vitro}, that doxycycline is not an appropriate clinical choice of antibiotic for the treatment of MRSP infections in the dog when tetracycline resistance is reported, even if doxycycline is reported as sensitive. Similar conclusions have been reported in humans where doxycycline resistance...
in both TetM- and TetK-positive methicillin resistant S. aureus isolates may be induced by incubation with subinhibitory concentrations of tetracyclines (TetM only for minocycline), suggesting that doxycycline may be a poor choice for any staphylococcal isolate exhibiting resistance to tetracycline by susceptibility testing. \(^7\) \(^2\) This is likely to be less of an issue in future testing as the proposed interpretative breakpoints of Maaland et al (2013) have been accepted by the CLSI and will appear in a future version of CLSI standards.\(^3\)

Where doxycycline is used for therapy in MRSP, it may be prudent to dose at the more ideal dose based on PK/PD testing of 5mg/kg q12h \(^1\) rather than the registered dose in Australia of a 5mg loading dose, two 2.5mg/kg doses 12h and 24h later then 2.5mg/kg q24h, to minimise the risk of resistance induction. For this reason also, the author will routinely treat MRSP cases with two antibiotics to which the infection is susceptible if systemic therapy is to be used.

Minocycline at the canine recommended dose of 5mg/kg q12h based on PK/PD testing \(^5\) may be an option in cases of MRSP resistant to tetracycline and doxycycline where the TetK and/or the much less common TetL genes may be present. Requesting minocycline sensitivity in MRSP cases where there is tetracycline resistance may provide a guide to these cases. However, current CLSI breakpoints for minocycline in staphylococcal infections are based on human data and likely too high for use in dogs \(^8\) but preliminary breakpoints have been suggested. \(^5\) Also, based on the only published study of MRSP genotypes in Australia it would appear the bulk of cases are associated with dru cluster 11a which is reported most often associated with a higher frequency of TetM resistance gene. \(^3\) If this is the case it would make minocycline less useful as a potential therapy for MRSP in this country. It is also possible though that the Australian isolates are more closely related to ST45 from Thailand, \(^9\) and if this is the case then the assumption above may not apply. The prevalence of TetK and TetM in Australian MRSP isolates requires further investigation.

Of interest in the two cases showing resistance to both tetracycline and doxycycline was the complete absence of any ZoI. In MRSA isolates, lack of inhibition around a 30ug tetracycline disc was indicative of the presence of both TetK and TetM genes. \(^2\) It could be speculated in this study the zero inhibition isolates may also carry both TetK and TetM, and the discordant results with more moderate levels of resistance and larger ZoI may carry TetK or TetM alone. Further investigation on ZoI on disk diffusion testing in combination with PCR for tetracycline resistance genes in MRSP is needed to clarify this.

The main limitations of the study were the relatively low numbers included, and the lack of clinical correlation with the reported in vitro results. Higher numbers of both would have added more power to the conclusion.

**Conclusion**

Doxycycline cannot be recommended for use in cases of canine MRSP where tetracycline is reported resistant based on current CLSI disk diffusion testing, irrespective of the result of doxycycline testing. Updates to these breakpoints in future CLSI editions may make this recommendation redundant.

**References**


Canine oral papillomavirus (COPV, CPV1) is a common virus in dogs. It is a contagious virus that typically enters through broken skin and is reported more frequently in young dogs and dogs on immunosuppressive medications. While most cases are mild and self limiting, occasional cases cause severe, non-regressing or recurrent infections. This has been reported to be likely secondary to defects in the host immune response rather than viruses of increased virulence. Treatment of non-resolving cases has been reported with withdrawal of immunosuppressive drugs, in addition to azithromycin, vaccines, and cryosurgery. Imiquimod (Aldara®, iNova Pharmaceuticals (Australia) Pty. Ltd) is a TLR-7 and 8 agonist which is registered in humans for the treatment of external genital and perianal papillomas, solar keratoses and basal cell carcinomas. Two cases are reported of COPV in dogs treated successfully using imiquimod.

Case 1:
A 1yo crossbreed dog was presented to ASES on prednisolone 0.5mg/kg q48h for pruritus of the face, ventrum, tail base and paws. Bacterial pyoderma with suspected underlying allergies was diagnosed and a diagnostic workup commenced including prednisolone reduction to 0.3mg/kg twice weekly, topical mometasone 0.1% daily for 7d then twice weekly, oral ivermectin to rule out scabies, an elimination diet trial and antibiotic therapy with chlorhexidine shampoo and clindamycin 450mg sid. At revisit after 67 days, pruritus was significantly improved but a single papillated nodule was noted on the left lower lip. Prednisolone was stopped. Revisit at d83 revealed a relapse in the pyoderma and bacterial conjunctivitis but also a 0.5-1cm papilloma on the left lower lip with 2-4 nearby smaller lesions and a similar single 2mm lesion on the left lower eyelid. In addition to clindamycin and topical ophthalmic fusidic acid, azithromycin 500mg sid was commenced for 10d. By d94 there were 3-4 large 0.5-1cm papillomas with four smaller ones on the left lower lip and 2 smaller lesions on the R lower lip. Three doses of azithromycin had been missed. Topical therapy with imiquimod topically was commenced using a thin smear on the larger warts external to the oral cavity on the L side only once daily for 7 days then three times weekly. By d109 there were 3-4 large 0.5-1cm papillomas with four smaller ones on the left lower lip and 2 smaller lesions on the R lower lip. Three doses of azithromycin had been missed. Topical therapy with imiquimod topically was commenced using a thin smear on the larger warts external to the oral cavity on the L side only once daily for 7 days then three times weekly. By d109 one a single papilloma remained and following another five treatments over the next week this lesion cleared and therapy was stopped. Prednisolone recommenced d110 and ASIT commenced d132 and there was no reports of relapse of oral papillomas at follow up at d180.

Case 2:
A 1yo Dalmatian was presented to ASES with a history of development of oral lesions after mouthing of a dead carp. There was no appreciable response to antibiotics and there was rapid regrowth of lesions following surgical debulking. Biopsy at this time confirmed oral papillomatosis. By d42 there were marked vegetative growths through the entire oral cavity. Treatment with azithromycin 10mg/kg q24, Cefovecin q14d, multiple short courses of meloxicam sid and a single episode of crushing some lesions over the next 63 days resulted in partial improvement. At presentation to ASES on d105 there were numerous papillomatous nodules on the perioral mucosa, buccal and gingival mucosa and the tongue. Imiquimod 5% was prescribed to be used on the large visible lesions external to the mouth five concurrent days per week for three weeks. At d131 at recheck there was 85% improvement and no new lesions seen. Isolated individual 4mm papillomas remained on the buccal mucosa and some confluent lesions on the anterior tongue still. Therapy was not continued. By d152 there was further improvement with just a few oral lesions reported. The case was subsequently lost to follow up.

Discussion
Resolution of canine papillomavirus is associated with antigen specific IgG and cell mediated responses. Imiquimod causes the activation of antigen-presenting cells (APCs) and acts as an immunologic adjuvant. By triggering cytokine production, imiquimod enhances the ability of APC to present viral or tumour antigens to reactive T lymphocytes, and amplifies type 1 helper T cell mediated immune responses.

The rapid response in both these cases suggests that the improvement in the papillomas was due to the imiquimod therapy but this cannot be confirmed – the small possibility that these cases represent spontaneous cure on lower
doses of prednisolone or eventual response to azithromycin cannot be ruled out. It is interesting in case one in particular that the papillomas appeared only after prednisolone reduction and continued to enlarge on lower doses.

In both cases the imiquimod was well tolerated. Inflammation secondary to therapy has been reported in humans, horses and cats\textsuperscript{10-12} but despite the higher than recommended frequency of application no adverse reactions were reported in these two cases.

Topical imiquimod should be considered for therapy of non-regressing canine oral papillomas.

References


Staphylococcal pyoderma

Bacterial folliculitis (other than dermatophilosis) is usually caused by a coagulase-positive Staphylococcus species. Both S. aureus and S. intermedium have been isolated. In one study, S. aureus accounted for twice as many isolates as S. intermedius; this study also isolated some strains of S. hyicus. Interestingly, in another study, lysozymes from equine neutrophils were only slightly bactericidal for S. aureus. Many isolates are resistant to penicillin G.

Occurrence of pyoderma has been linked to poor nutrition and husbandry in some cases. Recently, the staphylococcal species S. pseudintermedius and S. delphini were cultured from a skin lesion of a horse. Recent work would tend to suggest that what was previously termed S. intermedius is more correctly identified as S. pseudintermedius, and that S. delphini may be the most common staphylococcus species isolated from horses. Coagulase-positive staphylococcal species were not isolated from the skin of 20 horses admitted to a university teaching hospital for elective surgery.

Clinical signs

Clinical signs of staphylococcal pyoderma are most often crusts, usually in a circular pattern suggestive of dermatophytosis (this may be the reason that equine pyoderma is under-diagnosed), epidermal collarettes (circular skin lesions with an exfoliative border as seen in dogs with superficial pyoderma) or encrusted papules similar to the miliary dermatitis reaction pattern in cats. These infections tend to be variable in their intensity of pruritus. Histology usually shows folliculitis and/or furunculosis, but bacterial colonies are not always seen. A truncal form of bacterial folliculitis (contagious acne, contagious pustular dermatitis, Canadian horsepox) is often associated with poor grooming and trauma from tack and saddle, warm wet weather and heavy work. It is painful and interferes with working and riding. It is usually caused by a coagulase positive Staphylococcus species but may also be caused by Corynebacterium pseudotuberculosis, although this organism is more commonly a cause of deep pyoderma. Folliculitis often develops in the saddle and lumbar region, particularly in the summer. The affected area initially may be swollen and very sensitive; this is followed by formation of follicular papules and pustules. These may become confluent or rupture, forming plaques and crusts.

Another presentation is pastern bacterial infection (pastern folliculitis). Again, the causative agent is usually a coagulase-positive Staphylococcus species. The lesions are usually limited to the posterior aspect of the pastern and fetlock regions; one or more limbs may be involved. The initial lesions consist of papules and pustules. If left untreated, the lesions coalesce and may produce large areas of ulceration and suppuration, which may be quite painful. The disease is usually not associated with systemic signs and the general health of the horse is not affected. Important differential diagnoses are vasculitis, dermatophytosis, photosensitization (in white-coloured legs), chronic progressive lymphedema (in Belgians and other draft breeds), pemphigus foliaceus and chorioptic mange.

Deep pyoderma followed by ulceration may develop over large areas of the body, especially on the neck, sides of the thorax, inner surface of the thighs and axilla or on the prepuce. A relatively uncommon nodular variant termed ‘botryomycosis’ mimics actinomycosis or a deep fungal infection but is most often caused by Staphylococcus spp in the horse. These may require surgical excision as well as long-term antibiotics.

Public Health Considerations

In a 2000 study, methicillin-resistant, coagulase-negative staphylococci (MRCoNS) were cultured from healthy horses in Japan; the authors concluded, "These organisms must be considered a potential threat to horses and veterinarians who care for them." In a 2006 study from the Netherlands, MRCoNS were frequently found. The organism was usually S. sciuri, as opposed to S. epidermidis which was found in the humans in close contact with these horses. No methicillin-resistant Staphylococcus aureus (MRSA) was found in healthy horses. In a 2006 study from Slovenia, MRCoNS were isolated from the nares of 126/300 (42%) healthy horses. Again, no MRSA was isolated.

In contrast, a single strain of MRSA was isolated from both humans (13%) and horses (4.7%) on horse farms in Canada and New York state. MRSA was also isolated from 120 (5.3%) of 2,283 horses admitted to the teaching hospital at Ontario Veterinary College. Of these 120 horses, 50.8% were positive at the time of admission, and clinical infections attributable to MRSA were present or developed in 14. Horses colonized at admission were more likely to develop clinical MRSA infection. Administration of ceftiofur or aminoglycosides during hospitalization was the only risk.
factor associated with nosocomial MRSA colonization. Another strain of MRSA was isolated from a small number of horses at the Veterinary University, Vienna, Austria.

Of further concern is the finding of humans reporting skin lesions following contact with a community MRSA-positive affected foal, despite short-term contact with standard protective barriers. The isolates from the foal were indistinguishable from the ones from the humans. In a recent study, MRSA was isolated from 15.6% of veterinarians or technicians in large animal practice, as opposed to only 4.4% for small animal personnel; large animal practice was the only variable statistically significantly associated with colonization of MRSA. In contrast, the percentage of carriers in the horse care-taker community was only 2.4% in one study.

In two horse farms with endemic MRSA, active screening and strict implementation of infection control protocols resulted in a rapid decrease in number of colonized horses. The majority of horses eliminated MRSA without antimicrobial treatment. A recent study found that the MRSA strain USA 500, which predominates in horses and the people who work with them, is no more robust in attaching to equine keratinocytes than other strains.

A recent study from Italy concluded that harness racing-horses may act as a significant reservoir of MRSA as compared to breeding mares and riding-horses.

**Treatment of Equine Staphylococcal Pyoderma**

The antibiotic usually used for many bacterial skin infections in the horse is trimethoprim-sulfafurazol (30 mg/kg q12h for 2-6 weeks, longer for deep infections). Interestingly, dosing intervals for intravenous administration of trimethoprim-sulfamethoxazole in horses may not be appropriate for use in donkeys or mules. Donkeys eliminate the drugs rapidly, compared with horses.

In cases of *Staphylococcus* sp resistance to TMS, enrofloxacin or doxycycline may be used. Doxycycline is less expensive, but associated with a higher incidence of colic. Dosage is usually 10mg/kg q12h although a recent report suggests that 20 mg/kg q24 hr may also be used.

Off label usage of the oral enrofloxacin formulations for poultry, ruminants or swine has been suggested; a dose for the poultry formulation has been suggested as 7.5 mg/kg PO once daily. These formulations are not available in all countries. Use of enrofloxacin in young horses (less than 2 years old) should be avoided, due to concerns of damage to the articular cartilage. A report of the usage of an oral gel formulation of enrofloxacin (100mg/ml of gel) showed good clinical efficacy for infections in several organs; however, almost one-third of the horses had some diarrhea, and 10% developed oral lesions. The article stated that this latter side effect could be overcome with following administration with a tap water rinse of the oral cavity. However, the author has seen oral lesions and a swollen tongue, suggestive of a contact reaction, develop in a horse which was receiving the oral water rinse. Interestingly, enrofloxacin binds to melanin in equine hair, although the clinical implication is unknown.

Ceftiofur sodium (Naxcel®; Pfizer) 2.2 mg/kg, q12-24h, IM or IV, may also be used, although its usefulness over a long period is limited by its parenteral route.

In one report of 15 horses, vancomycin was used, alone or in combination with an aminoglycoside, to treat MRSA and enterococcal infections. The average vancomycin dosage was 7.5 mg/kg q8h given IV over 30 minutes. The antibiotic, alone or in combination with an aminoglycoside, was safe and effective. Because of the problems with emerging resistance, the authors recommended vancomycin use in horses be limited to cases in which culture and susceptibility indicate effectiveness and no reasonable alternative treatment is available.

For localized lesions, mupirocin ointment 2 (Muricin®, Dechra), or silver sulfadiazine cream (Silvadene®) may be effective. As shampoos ethyl lactate (Etiderm®, VIRBAC) or chlorhexidine (2%-4%) (TrizChlor4™, Dechra) are helpful. Recently, a gel with 0.4% stannous fluoride (MedEquine® Gel: Dolphin International, Inc, Camdenton, MO, USA) was reported as resolving bacterial skin infections in horses.

**Malassezia**

The exact species of this yeast growing on horses’ skin is just beginning to be investigated. In one study, the *Malassezia* sp. isolated were identified as *M. furfur*, *M. slooffiae*, *M. obtusa*, *M. globosa* and *M. restricta*. The author has examined several mares with a *Malassezia* infection between their mammary glands, which was intensely pruritic. The mares rubbed their tail and ventral abdomen. Physical examination showed a dry, greasy-to-the-touch exudate. Cytology of the exudate showed numerous yeast organisms, which were identified on culture as *Malassezia* species. Treatment with a topical 2% miconazole/chlorhexidine shampoo was curative. The author is aware of other similar cases. However, healthy non-pruritic mares may also have large numbers of yeasts in the intra-mammary area.
Alopecia areata

An idiopathic disease of horses (and other species) typified by areas of nonpruritic alopecia. Histologically, a lymphocytic infiltrate surrounding the base of the hair follicle is seen; however, in longstanding cases this infiltrate may not be present. A mare with a T-cell lymphocytic infiltrate causing a mural folliculitis of the isthmus (central section) of the hair follicles was described. Antibodies targeting the hair follicle itself have been documented. Lesions may be focal or progress to whole body alopecia (often sparing the mane and tail). One case was complicated by secondary Malassezia infection. Most horses regrow the hair but this may take up to two to three years. Corticosteroids may hasten the hair regrowth. Mane and tail “dystrophy” of Appaloosas and other breeds may in fact be a form of alopecia areata. This disease is also often mistaken for ringworm, due to the circular nature of the alopecia. In a recent review of 15 cases, a number of horses’ lesions waxed and waned often with the season.

Cutaneous vasculitis (aka pastern leukocytoclastic vasculitis, photoaggravated vasculitis)

This seems to be more common in California and the western United States (and doubtless, other regions with an excess of sunlight) as a clinical entity. It generally affects mature horses, and produces lesions confined to the lower extremities that (often but not always) lack pigment. Lesions are multiple and well demarcated with the medial and lateral aspects of the pastern the most common sites. Initially, erythema, oozing, crusting, erosions and ulcerations develop, followed by edema of the affected limb(s). Chronic cases may develop a rough or “warty” surface. The pathogenesis is uncertain: an immune-complex etiology has been suggested, and the limitation of lesions to nonpigmented areas suggests a role for UV radiation. Drug reactions may be a potential cause; a recent report implicated Staphylococcus intermedius. In a review of 72 cases, the most frequent clinical findings were crusts/scales and edema of the legs with the most common underlying disease being photo-aggravated dermatitis. Common laboratory findings were anaemia, neutrophilia, hyperglycaemia and hyperglobulinaemia. Histopathological patterns were most commonly cell-poor and lymphocytic/histiocytic. While statistically supported treatment recommendations could not be made due to the large numbers of confounding factors, trimethoprim-sulfa antibiotics, corticosteroids, and/or resolution or control of the underlying disease process were the most common treatment modalities. In some cases, avoiding sun exposure may be helpful.

Treatment is the use of corticosteroids at relatively high doses (1 mg/kg q12h of prednisolone, or dexamethasone 0.08-0.2 mg/kg q24h) for two weeks, then reduced over the next four to six weeks. Reducing UV light exposure is helpful, either by bandaging affected legs, stabiling inside during daylight hours, or both. In some cases, topical corticosteroids (such as triamcinolone spray 0.015% [Genesis™, Virbac]) may enable the horse to be weaned off of systemic corticosteroids. The author has also used pentoxifylline (10 mg/kg, q 12 h) as an adjunct treatment in one case, to good effect; another alternative (albeit more expensive) is the use of 0.1% tacrolimus ointment (Protopic®, Fujisawa) q 24h.

Equine sarcoidosis (also known as Chronic Granulomatous Disease [CGD])

This condition is characterized by skin lesions and occasionally widespread systemic involvement. This is an inflammatory disease and should NOT be confused with the neoplastic equine sarcoids. Equine sarcoidosis is infrequently encountered; in one report a predisposition for thoroughbreds and geldings was noted. Involvement of other organ systems (especially the gastrointestinal tract) portends a poorer prognosis.

To date, most recognized cases of equine sarcoidosis have been presented with a primary complaint of skin disease. The skin lesions commonly are generalized scaling and crusting associated with varying degrees of alopecia. Occasionally, the disease is focal or multifocal in distribution. Less frequently, the skin lesions consist of nodules or large tumor-like masses. The different types of skin lesions may co-exist. In addition to skin lesions, the most frequent presenting complaints are weight loss, decreased appetite, and a persistent low-grade fever. Lung involvement is manifested by exercise intolerance, increased resting respiratory rate, and mild dyspnea.

Regardless of the organ involved, the major histologic change is the presence of non-caseating granulomas consisting of aggregates of epithelioid cells and multinucleated giant cells. Neutrophils, lymphocytes, and plasma cells are present in variable numbers. In the skin, the granulomas tend to be located in the superficial portion of the dermis. The etiology of the disease has not been discovered. One report showed positive titers to Borrelia burgdorferi in three of four horses with GGD, another found evidence of equine herpes-virus and a recent report described mycobacterium DNA in the liver and skin of a horse with sarcoidosis. However, two recent reports using histopathologic stains, immunohistochemistry and/or polymerase chain reaction (PCR) assays on paraffin-embedded cutaneous specimens from eight horses were unable to obtain positive results for Mycobacterium spp., Coccidioides immitis, Cryptococcus neoformans, Corynebacterium pseudotuberculosis, Borrelia burgdorferi or equine herpesviruses 1 and 2. Because of the small number of horses studied and the variability of clinical signs, response to therapy has not been well documented. Immunosuppressive doses of corticosteroids have been helpful in the initial stages of the disease. A recent abstract reported that 5 of 6 horses did well and lived > 1 year with prednisolone treatment. Involvement of other organ systems (especially the gastrointestinal tract) portends a poorer prognosis. Recently
reported cases suggest that some horses with sarcoidosis may have localized rather than generalized skin disease, and confirm that spontaneous resolution (seemingly when the stress of working is reduced or eliminated) does occur.44, 45

References


Canine sterile nodular panniculitis – retrospective study

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Background: Canine sterile nodular panniculitis (SNP) is a sterile inflammatory disease of the panniculus that is typically managed with immunomodulatory or immunosuppressive treatments. It has been reported to be a cutaneous marker of underlying systemic disease.

Hypothesis/Objectives: To assess the presence or absence of concurrent systemic disease associated with canine SNP and document breed predispositions.

Animals: Thirty nine dogs presented to the veterinary teaching hospital from 1990-2012 that met inclusion criteria.

Methods: Retrospective study. Inclusion criteria: diagnosis of SNP via histopathologic analysis and negative special stains for infectious organisms and medical record available for review. Due to the retrospective nature, deep bacterial, fungal, and mycobacterial culture results were only available from 30, 19, and 5 dogs, respectively. The distribution of breeds was compared between dogs with a diagnosis of SNP and all other dogs examined at the same hospital, over the same time period, using an exact chi-square test of homogeneity.

Results: Australian shepherd dogs, Brittany spaniels, Dalmatians, Pomeranians, and Chihuahuas were significantly overrepresented. Thirty two dogs (82.1%) had no concurrent diseases, at either the time of initial presentation or during the available follow up period. The concurrent diseases in seven dogs included polyarthritis (four), long standing diabetes mellitus (one) and historical seizures (two).

Conclusions/Clinical Importance: To the authors’ knowledge, this is the first study to report a breed predisposition for SNP in Australian shepherd dogs, Brittany spaniels, Dalmatians, Pomeranians, or Chihuahuas. In this study, SNP was not typically associated with concurrent systemic illness.

Aspergillus Otitis – retrospective study

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Background – Aspergillus spp. Is a saprophytic opportunistic fungal organism and is one of the most common causes of otomycosis in humans. Two cases describing treatment and outcome have been reported in dogs but this is to the authors’ knowledge the first case series in dogs and cats.

Objective – To characterize the signalment, predisposing causes, case details, treatments and outcomes of dogs and cats diagnosed with Aspergillus otitis.

Animals – Eight dogs and nine cats diagnosed with Aspergillus otitis.

Methods – Restrospective review of medical records over a 25-year period of dogs and cats with a clinical diagnosis of Aspergillus otitis confirmed by culture and/or histopathology.

Results – Aspergillus otitis was usually unilateral and most commonly resulted in otitis externa and less frequently, otitis media. In dogs it was seen only in large breeds, greater than 23kg. Domestic shorthairs were the most common cat breed affected. The most common predisposing causes identified were underlying immunosuppression, otic foreign bodies and previous fluoroquinolone usage (topical or systemic). Otic lavages under anaesthesia and/or surgical intervention increased the frequency of resolution. Aspergillus fumigatus was the most common isolate and was more common in cats. Aspergillus niger and Aspergillus terreus were also cultured from dogs.

Conclusions – Aspergillus otomycosis is an uncommon cause of unilateral otitis externa in in cats and primarily large breed dogs. This diagnosis should be considered in animals with a history of immunosuppression, otic foreign bodies and previous fluoroquinolone usage.

Feline Pemphigus Foliaceous –retrospective study

This study is still in its early stages. We have 40 cases that we are evaluating – 3 in just the last 4 months. Early impressions: cyclosporine was not that helpful in controlling the disease: all the cats we started on Atopica ended up...
needing corticosteroids as well. Many (number to be determined) of the cats started on just corticosteroids were able to go into remission.

A new medication to treat canine pemphigus foliaceus
The goal of this study is to assess the efficacy and safety of a Bruton’s Tyrosine Kinase inhibitor (BTKi) as a therapy for canine pemphigus foliaceus (PF). Only 3 dogs treat so far: one had an excellent response, two are still in the early stages of the trial. Whether this drug will be brought to market is unknown.

Equine Hyperesthetic Leukotrichia – retrospective study
Also in early stages. Literature suggests quarter horse predilection, but our cases (around 18) had Arabians as the primary breed and only a few Quarter Horses. Many of them have a recurrence seasonally and there are a few cases where there were multiple horses in the barn affected concurrently. Despite theory of reaction to rhinopneumonitis vaccine, on first examination of records, that does not seem to be a trigger, although one horse’s disease was temporally related to being vaccinated for West Nile virus.

Fluconazole and alopecia – prospective study
Fluconazole in people is the medication used most commonly to treat infection with Coccidioides imitis (valley fever) but occasionally causes alopecia. This also seems to be the case in rats. In co-operation with the UC Davis School of Medicine, we are evaluating rats for changes in the anagen/telogen ratio (both histologically and using trichoscopy) when they are given fluconazole. That fluconazole adversely affects the hair cycle in rats has been shown in a paper from Poland in 2013.