CANINE
DERMATOLOGY

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COMING UP...

In our next issue, we will look at various aspects of nutrition.

- **Canine weight management**
  Alex German, UK

- **Potential deficiencies and problems in homemade diets**
  Marge Chandler, UK

- **The canine microbiome**
  Jan Suchodolski, USA

- **Feline nutrition – Q and A**
  Ana Luisa Guimarães Dias Lourenço, Portugal

- **Feline nutrition – common myths and misconceptions**
  Karolina Hołda, Poland

- **Grain free diets and cardiomyopathies**
  Joshua Stern and Jennifer Larsen, USA

- **Calcium and phosphorus metabolism in dogs**
  Linda Böswald and Britta Dobenecker, Germany

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CYNICISM IN THE 21ST CENTURY

"Υπάρχει μόνο ένα καλό, η γνώση και ένα κακό, η άγνοια" – **Socrates** (There is only one good, knowledge, and one evil, ignorance)

Diogenes of Sinope, who lived some 23 centuries ago, was not, perhaps, a particularly likeable person. According to various sources he spent many years as a beggar on the streets of Athens, living a life of austerity and sleeping in a huge clay jar that was open to the elements. A near contemporary of Socrates, he made a virtue of extreme poverty, scorning conventional values of the time and declaring contempt for all human achievements, social values and institutions. As a founder, and the archetypical practitioner, of the ancient Greek philosophical school of Cynicism, he held that people are motivated purely by self-interest rather than acting for honorable or unselfish reasons. Diogenes and his fellow Cynics believed that the true goal in life is mental clarity and lucidity, which brings freedom from false beliefs, mindlessness or folly.

Interestingly, in English the word "cynic" is derived from the Greek word kynikos (or "dog-like"), and it is possible that the Athenians originally used the word as a derogatory epithet for those who subscribed to Cynicism. However, Diogenes and his fellow Cynics may have regarded such a comparison to be complimentary; they could have pointed out that – amongst other things – dogs are good guardians of their principles, they are loyal and dependable, and are able to discriminate between friends and enemies without prejudice.

Of course, the concept of cynicism has come down through the ages, so that we now employ the term if we perceive someone who appears to be motivated by ambition or greed, or something that is vain, unobtainable, or ultimately meaningless. But while the Cynics of old could claim that this issue of *Veterinary Focus* is certainly dog-like – concentrating as it does on canine dermatology – there is no way that they could fault it otherwise, as it contains articles that are rational, lucid and self-enlightening. The contributors who have authored this issue can be confident that their efforts will not be regarded with cynicism, suspicion or distrust by our readers.

Ewan McNEILL
Editor-in-chief

In this issue of *Veterinary Focus*

THE DIAGNOSTIC CHALLENGES OF CANINE ATOPIC DERMATITIS

Clinicians face a challenge when confronted with a possible case of canine atopic dermatitis; Ana Rostaher reviews the diagnostic options and offers some practical tips.

KEY POINTS

1 The diagnosis of canine atopic dermatitis is problematic, as there is currently no reliable biomarker that can distinguish the disease from other dermatological disorders.

2 When faced with a possible atopic dog, the clinician must interpret and consider various aspects, including the patient's history, characteristic clinical features and exclusion of other differential dermatoses.

3 Intradermal testing (IDT) is the preferred diagnostic method among dermatologists to identify canine atopic dermatitis and ascertain the causal allergens.

4 Allergen-specific IgE serology offers several advantages over IDT and is often used as an alternative for canine atopic dermatitis diagnosis, but there are also disadvantages, with false positives being a potential risk.

Introduction

Canine atopic dermatitis (CAD) is a common inflammatory skin disease, affecting up to 15% of the global dog population (1). The pathogenesis of the disease is multifactorial, with both skin barrier dysfunction and immunological dysregulation known to have central roles, and both may be influenced by genetic and environmental factors. IgE and non-IgE mediated immunological events are key features in the pathogenesis, with allergens constituting the main triggering factors (2). The most commonly associated laboratory feature in CAD is the allergen-specific serum IgE levels, but (in contrast to humans) elevated total IgE levels do not assist in the diagnosis of CAD. Dogs are reported to have much higher levels of IgE than humans, probably as a result of their more frequent exposure to parasite infestation (3).

There are two major risk factors for atopic dermatitis; breed predisposition (*e.g.*, 50% of West Highland White terriers may be affected) and a familial history of CAD (4). However, since both genetic and environmental factors are involved, the phenotypic manifestation of the disease is highly variable – not only between different breeds, but also among individual dogs of the same breed. Given that CAD is both a complex disease with multiple facets and that other skin conditions may mimic the condition, a definitive clinical diagnosis is considered challenging.

Diagnostic considerations

Because there is currently no reliable biomarker that can distinguish CAD from other dermatological disorders, the diagnosis of CAD remains clinical, and hence the clinician must interpret and consider various aspects, including the patient's history, characteristic clinical features and exclusion of other differential dermatoses. **Figure 1** offers a workflow for the diagnosis of CAD. The first step is to rule out other CAD-mimicking diseases, because although pruritus is the most consistent finding, it is by no means exclusive for CAD, and other differentials should be considered. Ectoparasite infestations or bacterial or yeast infections, secondary to a non-pruritic disorder (*e.g.*, endocrinopathies, sebaceous adenitis), or less frequently neoplastic disease (*e.g.*, cutaneous lymphoma, though more commonly seen in older patients), should be ruled out during the initial workup phase on the basis of the signalment, history or additional targeted tests **(Table 1)**. It is worth noting that one aspect very typical for CAD may be observed at the onset, when pruritus may be alesional or associated with primary skin lesions such as erythema and sometimes papules. With progression over time and additional secondary infections, signs such as pustules, alopecia, excoriations, lichenification, crusting and seborrhea may develop. The face, inner aspect of the pinnae, axillae, abdominal, inguinal and/or perineal areas

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and distal extremities are typical predilection sites in most dogs with CAD **(Figure 2)**, although the affected body areas may vary with breed (5).

Once other potential etiologies have been ruled out, the standardized clinical criteria for CAD ("Favrot's criteria") can be applied to aid interpretation of the clinical findings in a pruritic dog **(Table 2)**. These should not be employed before this point, because whilst ~80% of dogs that fulfil five of these criteria will have CAD, the remaining 20% will have another disease. Conversely, around 20% of dogs that do have CAD will not demonstrate at least five of these factors.

Testing for environmental allergens

Once a clinical diagnosis of CAD has been made, further assessment is indicated, particularly to determine which allergens exacerbate clinical signs. This approach enables both appropriate selection of avoidance measures (especially with food allergens, although some measures can also be taken against house dust mites) and selection of allergens for allergen-specific immunotherapy. In general, if a dog has seasonal CAD, an immediate work-up for environmental allergens is warranted, but for cases with perennial pruritus and/or gastrointestinal clinical signs, food-induced dermatitis should be excluded before testing for environmental causes. An approach often used by the author is to initiate feeding of a commercial hydrolyzed diet using an elimination diet protocol. If the clinical signs of CAD persist despite this, testing for environmental allergens is followed, either by *in vivo* skin testing (most commonly intradermal testing, or IDT) or *in vitro* allergen-specific IgE serology (ASIS). Other than a poor response to a dietary trial, factors that would prompt allergy

testing would be if a dog has severe clinical signs, where signs persist for more than 3 months each year, or if management with symptomatic therapy is unsuccessful (either because of significant drug side effects or poor owner compliance) (6).

It must be stressed that neither IDT or ASIS is a screening test for CAD; they only assist in confirming the clinical diagnosis and identification of allergens. Most dogs with CAD will have allergen-specific IgE to environmental allergens identified on testing, although in some cases IgE levels are not elevated ("atopic-like dermatitis").

Both tests have their limitations and advantages, with neither being superior, and since the success rate of allergen-specific immunotherapy (ASIT) suggests that the two methods deliver comparable results (7) they may therefore be regarded as complementary. The author therefore prefers performing both skin testing and ASIS if costs allow, although if the former presents potential

Table 1. Additional testing methods used in a CAD work-up to assess for any concomitant or atopic dermatitis-resembling disease, in addition to an elimination diet trial.

Flea combing	Fleas
Skin cytology	*Malassezia* dermatitis Bacterial dermatitis
Skin scrapes/ hair plucking/ tape stripping	Scabies Other ectoparasites: *Demodex* spp., *Cheyletiella* spp., *Neotrombicula autumnalis* Dermatophytosis
Fungal culture	Dermatophytosis
Skin biopsy	Sebaceous adenitis Cutaneous lymphoma

STEP 1
Rule out conditions that can mimic CAD

STEP 2
Apply Favrot's criteria
(Table 2)

STEP 3 (Optional)
Apply specific clinical criteria
(Table 2)

STEP 4
Allergen tests to confirm the offending allergen

Figure 1. The four steps in the diagnostic approach to CAD; a patient should always be worked up in this order. Step 3 (specific criteria) should be used only where Favrot's criteria are not diagnostic, but the suspicion of CAD is high.
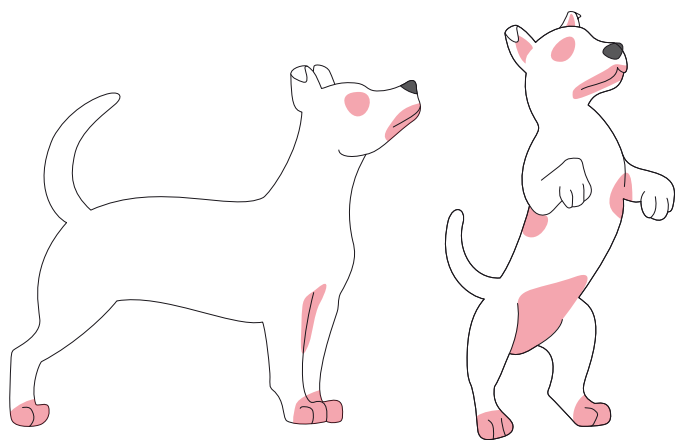
Figure 2. The red coloration depicts the most typical predilection sites for canine atopic dermatitis.

Table 2. Clinical criteria for diagnosing canine atopic dermatitis.

Favrot's criteria – the 8 major indicators for CAD (from 5)	
History	**Clinical exam**
• Onset of signs under 3 years of age • Dog living mostly indoors • Glucocorticoid-responsive pruritus • "Alesional" pruritus at onset	• Affected front feet • Affected inner pinnae • Non-affected ear margins • Non-affected dorso-lumbar area
Specific clinical criteria for CAD	
Additional body sites which might be affected • Lips • Eyelids • Ears (outside) • Dorso-lumbar region • Thorax • Flexural body regions **Recurrent skin/ear infections**	

risks, or the patient is uncooperative, ASIS should be the initial option. If the two methods produce inconclusive results, the results are combined for ASIT, otherwise the choices for ASIT are generally based on the ASIS results. Importantly, for either method clinically relevant allergens must be chosen, which is very much dependent on the patient history and clinician's judgement.

In addition, skin prick testing has recently become fashionable again, although as yet it is unvalidated in veterinary medicine. Saliva testing is also becoming commercially available, but at the time of writing it cannot be recommended as a diagnostic tool.

Intradermal testing (IDT)

IDT is an indirect measure of cutaneous mast cell reactivity, based on the presence of allergen-specific IgEs on their surface, and is the preferred diagnostic method among dermatologists, partly because mast cells can bind individual allergen-specific IgE molecules for more than a year (8). Data on the

sensitivity and specificity of IDT is scarce, although literature reports suggest it to be 30-90% and > 50-95% respectively (6,9). However, a precise assessment is very difficult due to a large number of both intrinsic factors (*e.g.,* patient immunologic make-up) and extrinsic factors (*e.g.,* allergen quality, skill level in performing IDT, season, medications).

Allergen selection

The selection of the most relevant allergens to be used for IDT depends on the animal's geographic location, and may be aided by resources such as specialized veterinary and human clinics, allergy laboratories, textbooks and the relevant national allergy bureau. Nevertheless, the choice should be reviewed periodically, with individual allergens removed or incorporated as appropriate. For example, the author's initial IDT panel, consisting of 43 allergens, has been reduced to the most frequently found 13 environmental allergens **(Box 1)**, and is aligned with allergens used in the local human dermatology clinic. This revised panel has shown no reduction in the efficacy of ASIT over a seven-year period.

IDT can utilize either lyophilized allergens or pre-diluted aqueous allergens intended for immunotherapy (which usually have a shelf life of at least 6-12 months), with the allergens further diluted as indicated in **Table 3**. They remain stable for up to 2 weeks if stored at 4°C in plastic syringes, or 8 weeks in glass vials, but otherwise allergen extract potency deteriorates with time (9), dilution and higher temperatures. Glycerinated allergens (usually used for prick tests in humans) should be avoided due to the possible irritative effects of the glycerin preservative.

Methodology

The only currently available recommendation for the optimal timing for IDT in dogs with seasonal disease is to test at the end or within 2 months of the peak season (10); this avoids possible peak season anergy or out-of-season low IgE levels, although some dogs may show sufficient IDT reaction if tested during their peak season. Dogs with non-seasonal disease may be tested at any time of the year.

IDT can be performed on non-sedated dogs, standing (the author's preferred option) or in lateral recumbency. Some sedatives are said to negatively influence the IDT results (*e.g.,* oxymorphone, ketamine/diazepam, acepromazine and morphine)

Box 1. The author's current choice of 13 allergens for intradermal testing.

- House dust mites: *Dermatophagoides farinae, Acarus siro*
- Pollens
  - Grasses: *Phleum pratense, Dactylis glomerata, Secale cereale*
  - Trees: *Fraxinus* spp., *Betula* spp.
  - Weeds: *Rumex crispus, Chenopodium album, Plantago lanceolata, Ambrosia* spp., *Artemisia vulgaris*
- Yeasts: *Malassezia* spp.

and should be avoided whenever possible, whilst others (*e.g.*, xylazine hydrochloride, medetomidine (dexmedetomidine), tiletamine/zolazepam, thiamylal, halothane, isofluorane, and methoxyfluorane) can be used safely (6). Recommendations on the use of propofol for IDT are still controversial and therefore its use is not currently recommended. Importantly, withdrawal times for some medications (which can lead to false negative results) should also be considered **(Table 4)**.

The skin site (usually the lateral thorax) is gently shaved (with the size depending on the number of allergens to be used) but should not be scrubbed or washed. Individual test sites are marked with a waterproof marker placed at least 2 cm apart, and a small volume (typically 0.05 mL) of each test concentration injected intradermally **(Figure 3a)**. A skin bulge should appear; if absent, the allergen has been applied too deeply (subcutaneously) and the injection should be repeated.

The reactions are evaluated after 15-20 minutes, with any wheal and erythema formation at each site compared to the positive and negative controls **(Figure 3b)** and scored, from 0 (equal to the negative control) to 4 (equal to the positive control). Any reaction of 2 or greater is regarded as positive. Although the assessment can be done objectively (by measuring the diameter of the reaction) no definitive benefit has been noted for this option (6) and the author prefers to simply assess the reactions subjectively.

Adverse reactions to the test are rare; if they do occur it is predominantly during the actual procedure, usually as an intense pruritus at the injection site (local hypersensitivity reaction) which can be alleviated by a short course of topical glucocorticoids or systemic anti-inflammatory or anti-pruritic treatment. Rarely, other events such as anaphylaxis (generalized itching, vomiting, diarrhea or even collapse) can develop, and should be addressed appropriately.

Allergen-specific IgE serology (ASIS)

In vitro ASIS is widely used in veterinary medicine as it offers several advantages over IDT. These include elimination of life-threatening risks for the patient (related to sedation or anaphylactic reactions), convenience (no hair clipping, no restraint, short duration) and a lower likelihood of prior or current drug therapy adversely influencing results (9). Various tests are available, either as solid phase RAST or ELISA methods (the latter being the most frequently used) or as a liquid-phase immunoenzymatic assay (9). When first introduced, these IgE tests demonstrated some disadvantages, especially poor specificity. Various improvements, particularly with the development of appropriate anti-canine IgE detection reagents, has improved their diagnostic accuracy (11). Other limitations of ASIS are the potential for inter-and intra-laboratory variability and cross-reactivity (12). Furthermore,

Table 3. Reported allergens and recommended concentrations for canine IDT*.

Allergens	Published concentrations/dilutions
Pollens	1000 to 8000 PNU**/mL
Molds	1000 to 8000 PNU/mL
House dust mites	
D. pteronyssinus	100–200 PNU/mL
D. farinae *Tyrophagus putrescentiae* *Lepidoglyphus destructor*	75 PNU/mL
Acarus siro *Blomia tropicalis*	50 PNU/mL
Epidermal extracts	At least 1,250 PNU/mL 300 PNU/mL for human dander
Whole flea extract	1:500 w/v

Table 4. Drug withdrawal times before allergen testing.

Drug name/class	IDT*	ASIS***
Antihistamines	7 days	Probably not needed
Short acting glucocorticoids	14 days	Not needed
Long-acting injectable glucocorticoids	< 28 days	< 28 days
Topical glucocorticoids	14 days	Not needed
Cyclosporine	Probably not needed	Not needed
Oclacitinib	Probably not needed	Probably not needed
Lokivetmab	Not needed	Not needed
Pentoxyfilline	Not needed	Not needed

* IDT: Intradermal testing
** PNU: Protein Nitrogen Units
*** ASIS: Allergen-specific IgE serology

"Once a clinical diagnosis of canine atopic dermatitis has been made, further assessment is indicated, particularly to determine which allergens exacerbate clinical signs."

Ana Rostaher

Injection of allergens

Figure 3. The intradermal testing procedure. **(a)** An insulin syringe with a fine needle (30 G, 8 mm) is used to inject 0.05 mL of the diluted allergen intradermally (not subcutaneously); correct placement is signified by a small "bulge" in the skin.
(b) The reactions are read after 15 minutes; here four of the allergens produced positive erythema and wheal formation (arrowed) comparable to the positive (+) control (score = 4). The negative (-) control can also be seen.

Interpretation of results

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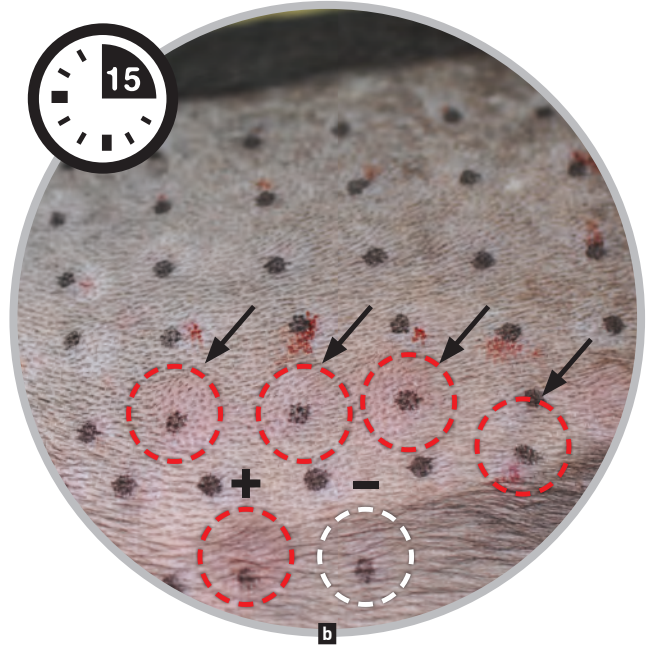
recent data show that the presence of IgE antibodies against cross-reactive carbohydrate determinants (anti-CCD antibodies) may be partially responsible for false positive results, especially with pollens (13). Blocking anti-CCD antibodies has resulted in a markedly improved correlation between IDT and ASIS in dogs (12) and a notable decrease in positive reactions to pollen allergens in cats (14). Clinically relevant is the fact that the results obtained with ASIT do not appear to depend on the choice of ASIS methodology (9) – and as noted above, ASIT efficacy is comparable whether the choice of allergens is based on IDT or ASIS results. Because of this, ASIS may be the preferred diagnostic choice for first-line clinicians where IDT is not an option, either in-house or via referral to a specialist center.

●●● Other testing options

Skin-prick testing is the method of choice for detection of Type I hypersensitivity in human atopic dermatitis, for several reasons; low allergen costs (glycerinated allergens tend to be stable for prolonged periods of time), rapid interpretation of results, absence of side effects, and high specificity (15). It is also said to be significantly less painful.

One report on prick testing in veterinary allergology dates back to the 1990s (16), but it concluded that the method was inferior to IDT in terms of result interpretation, and no further attempts were made to bring this test into clinical practice. However, within the last few years renewed clinical and

scientific interest has developed to assess the benefits of this diagnostic tool in dogs and cats. In one study the test was performed in 20 healthy dogs with 8 different environmental allergens (17), with no signs of pain or discomfort noted during the simple procedure (which took on average 5 minutes, including hair clipping and allergen application). The intensity of positive results ranged from 3-12 mm (median 9 mm), but this study only assessed threshold values in healthy dogs. A similar study assessed the sensitivity and specificity of this method on 11 common environmental allergens in both non-allergic dogs and dogs with spontaneous atopic dermatitis (18). The sensitivity was estimated to be 66% (the offending allergens could be identified in 3/5 dogs, with false negative results in the other two dogs) and 100% specificity (no dog had false positive results). Although yet to be validated in veterinary allergology, such studies suggest that prick testing might in future be a practical, accurate method that could be used as an important adjunct diagnostic for CAD. The author currently uses this test mainly to verify severe hypersensitivity reactions to *Hymenoptera* (*e.g.*, bees and wasps) venom (19), with the procedure shown in **Figure 4**.

Lastly, various saliva- and hair-based assays for the diagnosis of adverse food reaction (AFR) and/ or environmental allergies are now available in some countries. However, recent studies in dogs showed a lack of sensitivity and specificity for any of these tests (20-22), and so their use is discouraged at least for now.
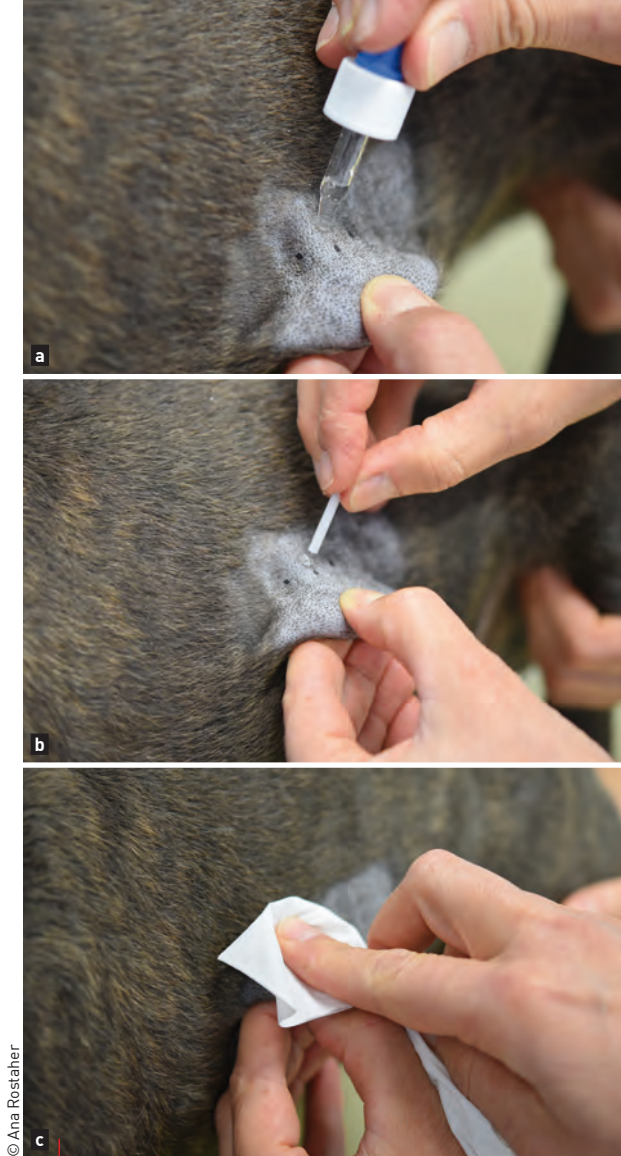
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Figure 4. Performing a skin prick test in an atopic dog for the house dust mite allergen *Dermatophagoides farina*. This can be done without sedation, with the dog in a standing position and the flank shaved as for an IDT. **(a)** One drop of the environmental allergen is applied to the skin. **(b)** The skin is immediately pricked using a commercial device held at 45⁰ to the skin. **(c)** The remaining fluid is removed with a clean paper towel and the procedure repeated for the other allergens. Positive and negative controls are applied in the same way, and the test is read (as for IDT) after 15 minutes.

CONCLUSION

The diagnosis of atopic dermatitis can only be made on the basis of data derived from the patient's history, clinical examination and by ruling out other differential diagnoses. No laboratory test can diagnose canine atopic dermatitis and therefore its over-utilization should be discouraged to limit misdiagnosis. Identification of the causative allergen in atopic dermatitis is the essential last step in the work-up, significantly influencing the long-term management and quality of life of the patient.
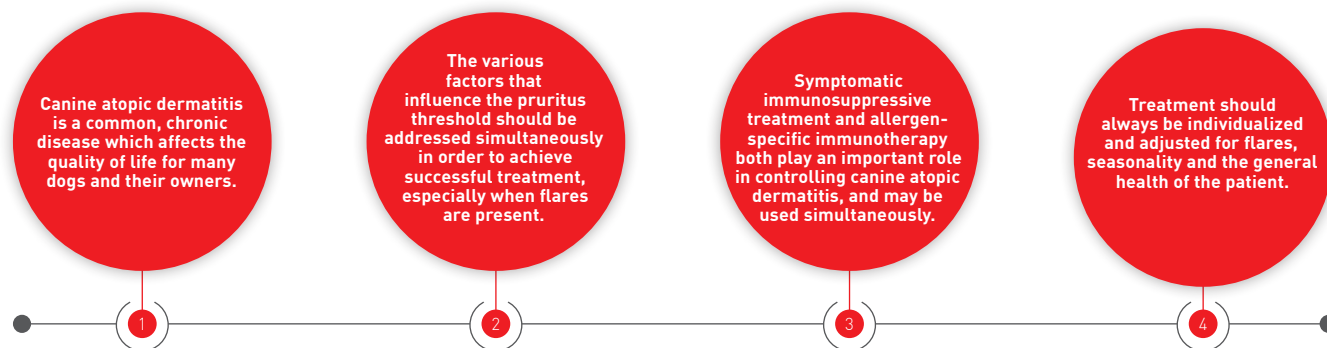
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TREATING CANINE ATOPIC DERMATITIS

The atopic dog is an all-too-often presentation in first opinion clinics; this paper reviews the options for treatment and emphasizes the need for a multi-modal approach.

KEY POINTS

1 Canine atopic dermatitis is a common, chronic disease which affects the quality of life for many dogs and their owners.

2 The various factors that influence the pruritus threshold should be addressed simultaneously in order to achieve successful treatment, especially when flares are present.

3 Symptomatic immunosuppressive treatment and allergen-specific immunotherapy both play an important role in controlling canine atopic dermatitis, and may be used simultaneously.

4 Treatment should always be individualized and adjusted for flares, seasonality and the general health of the patient.

Introduction

Canine atopic dermatitis (CAD) is a common allergic skin disorder that develops from predominantly environmental allergens, such as house dust mites and pollens of grasses, trees and weeds. The etiology is considered multifactorial, whereby an epidermal barrier dysfunction, combined with dysregulation of the immune system, leads to the development of clinical disease in dogs with a suggested genetic background of CAD. In most cases the problem starts at a young age, but causes discomfort by dermatitis and pruritus throughout life.

Several therapies have been developed for CAD, but each has its pros and cons regarding effectiveness and health interference. This article offers a logical approach to the daunting question "where do we start?" The challenge is not only to treat the patient successfully, but also to avoid severe flares. For this reason, the treatment of CAD requires multifaceted management **(Figure 1)**. Successful remission can only be achieved with a combination of approaches in order to control the clinical signs and prevent flares, and the options will depend on a patient's individual needs and the severity of disease over time.

Avoidance of allergens

Since the most common causal allergens are house dust mite glycoproteins and aeroallergens, such as pollens, prevention of allergen contact is difficult or impossible to achieve. An uncontrolled study that

used an environmental benzyl benzoate acaricide spray in an attempt to reduce the amount of dust mite allergens reported some clinical improvement in atopic dogs (1). Other environmental sprays currently also marketed for the human field contain probiotics which produce enzymes for isolating house dust mites' fecal proteins. Further controlled studies are needed to clarify the correlation

Figure 1. The multimodal treatment and management of canine atopic dermatitis.

Avoidance of allergens

Repair the skin barrier

Alter the immune response by ASIT* (SCIT** or SLIT***)

Control inflammation and pruritus

Control skin infections
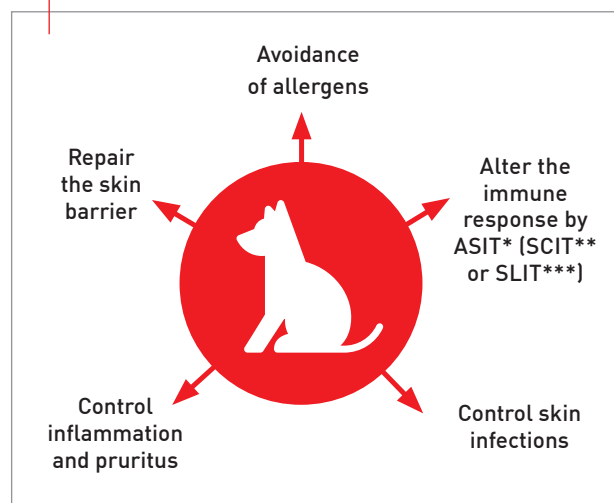
* Allergen-specific immunotherapy ** Subcutaneous immunotherapy
*** Sublingual immunotherapy

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of clinical improvement in atopic dogs and the reduction in dust mites allergens with these sprays. Likewise, the use of dust mite-free mattresses, regular vacuuming, and washing blankets at 60°C is also likely reduce the canine skin's exposure to house dust mites allergens.

Rarely, an atopic dog may be responsive to epithelia of other pets in the household (*e.g.*, parrot or guinea pig). In this situation it is advisable to relocate either the causal pet or the patient to another household.

When food-induced atopic dermatitis is present, both food allergens and environmental allergens play a causal role (2). Food allergens may especially be significant when flares occur, and determining the role of food with an elimination diet trial and provocation is always essential for the atopic dog; if proven, prevention of exposure to causal food allergens is often relatively easy to establish.

Repairing the skin barrier

It is well known that atopic dogs suffer from an impaired epidermal barrier, which results in an increased transepidermal water loss (TEWL). Dry and scaly skin (xerosis) may be seen in some breeds. Supporting the epidermal barrier with topical moisturizers such as glycerol, glycerin, propylene glycol, panthenol and urea will increase the water-binding capacity of the epidermis, especially when used after bathing. This has been recently demonstrated in a chronically disrupted canine epidermal barrier model (3). Products containing phytosphingosine and ophytrium, a natural ingredient extracted from the root of the Japanese mondo grass plant, may also help improve the skin barrier and reduce pruritus and colonization of microbes at the epidermis (4).

Atopic dogs also have disrupted intercellular lipid lamellae of their stratum corneum. To restore this, oral essential fatty acids (EFAs), either as supplements or incorporated in the diet, have been deployed with varying results. Of interest is one good quality study which showed a significant reduction in the required prednisolone dosage in atopic dogs when oral EFAs were administered for 12 weeks (5). Alternatively, it is possible to use a complete diet containing compounds that offer skin barrier support. Topical EFAs in a spot-on formulation have also been proven to be effective (6), although this option may be less cost-effective if long-term application is required. Other topical

formulations including shampoo, sprays, and lotions containing fatty acids and ceramides have been introduced for CAD. Unfortunately, there is still some inconsistency in the effectiveness of these products, but the clinician should bear in mind that by restoring the epidermal barrier, skin penetration by environmental allergens is probably reduced.

Controlling secondary skin infections

Most atopic dogs are prone to recurrent superficial pyoderma, and papules, pustules, collarettes, squames, and seborrhea are commonly seen **(Figure 2)**. Colonization of the atopic skin by pathogenic *Staphylococci* spp. (usually *S. pseudintermedius*) is increased compared to healthy skin, which may be partly explained by lower antimicrobial activity of the cutaneous antimicrobial peptides of the innate immune system. During flares, dysbiosis of the atopic skin microbiota develops, with a relative increase in *Staphylococci* levels. This dysbiosis is restored with antimicrobial therapy and the remission of lesions (7).

About 40% of atopic dogs have recurrent skin infections with the yeast *Malassezia pachydermatis,* with a strong odor, greasiness, honeycomb crusts, squames, and paronychia with brown staining of the nails often noticed **(Figure 3)**. A type I hypersensitivity reaction to *Malassezia* can also

Figure 2. Atopic skin with classic lesions – papules, pustules and collarettes due to secondary superficial pyoderma.

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Figure 3. Paronychia in an atopic dog with brown staining of the nails due to *Malassezia* dermatitis.

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Figure 4. Interdigital pyogranuloma with secondary deep pyoderma in an atopic dog.

occur, leading to severe pruritus (8). Secondary skin infections by bacteria and yeasts must therefore always be controlled, and is achieved by regular use of topical antimicrobial therapy (shampoos, mousses, sprays, wipes and gels). Shampooing with 3% chlorhexidine has been shown to be clinically as effective against bacteria and yeasts as a 2% chlorhexidine solution and miconazole combination (9). Twice weekly washes are generally efficacious, but (depending on the severity of the infection) topical therapy should be administered more frequently initially. The author then uses daily washings for a week, followed by a week of every other day, and then twice a week. Protocols that include twice weekly application of a mousse, gel, or spray on the lesions, in addition to weekly shampooing, seem to work equally well.

Systemic antibiotics should only be used initially when the pyoderma is deep (*e.g.*, with furunculosis **(Figure 4)**, very generalized, or when the owner cannot treat the dog topically. When selecting an

appropriate drug, this may either be done after culture and susceptibility testing, or following the basic principles of antibiotic therapy; options include clindamycin (10 mg/kg q12H), cephalosporins (cephalexin 10-30 mg/kg q8-12H), or clavulanic acid-potentiated amoxicillin (12.5 mg/kg q12H). Always treat until both clinical signs and cytological findings of pyoderma have resolved. Recurrent use of antibiotics should be avoided because of the risk of inducing bacterial resistance. Likewise, oral treatment with ketoconazole (10 mg/kg q24H or 5 mg/kg q12H) or itraconazole (5 mg/kg q24H) for yeasts should only be used in very severe cases, as yeasts can (rarely) become resistant to azole derivatives (10). However, remember that (especially with ketoconazole) various undesirable drug interactions are possible.

Controlling skin inflammation and pruritus

Symptomatic therapy that has good evidence for reducing pruritus and dermatitis in CAD includes glucocorticosteroids, cyclosporine, oclacitinib, and lokivetmab, and these will be discussed in turn. Note that preventive flea treatment is always essential to reduce the itch threshold. There is no conclusive evidence that oral type-1 antihistamines effectively treat either active or chronic CAD lesions (11), but if required the best options are cetirizine (0.5-1.0 mg/kg q24H) or hydroxyzine (2 mg/kg q12H) (12).

Glucocorticosteroids

Glucocorticosteroids (GC) are effective through interference with multiple ubiquitous transcription factors leading to repression of genes coding for cytokines, cytokine receptors, adhesion molecules, pro-inflammatory enzymes, and chemotactic proteins. They therefore deactivate many inflammatory cells and reduce the itch, and as they are fast-acting in nature they can be used both

"The challenge is not only to treat the canine atopic patient successfully but also to avoid severe flares, and for this reason the treatment of CAD requires multifaceted management."

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to induce remission of acute signs and maintain long-term control in CAD. However, because they impact on many different cellular mechanisms side effects are common with prolonged systemic administration; these include polyuria, polydipsia, polyphagia, muscle and skin atrophy, susceptibility for bacterial and fungal infections, demodicosis, and iatrogenic hyperadrenocorticism **(Figure 5)**. Parenteral formulations should therefore not be administered repeatedly, but short-acting glucocorticoids are recommended when clinical signs are severe. Oral prednisolone (0.5-1 mg/kg q24H) or methylprednisolone (0.4-0.8 mg/kg q24H) should be administered for 5 to 14 days, based on the patient's response to treatment. Dividing the dose twice daily may reduce polydipsia and polyuria in some individuals. The dose may then slowly be tapered and administered every other day as the clinical signs reduce.

The preferential GC formulation is a topical ointment, spray or lotion. Both triamcinolone acetonide and hydrocortisone aceponate sprays show high efficacy in bringing localized lesions under control (13); these should be used every day for about two weeks to induce remission and then continued on individual lesions as often as twice weekly. Hydrocortisone aceponate could potentially induce mild dermal degradation through inhibition of collagen I and III pro-peptides, but one study showed no visible skin atrophy during long-term intermittent (twice weekly) topical application in CAD cases (14). Human preparations such as betamethasone or mometasone furoate creams have also shown to be effective in veterinary practice. The ultimate goal of maintenance treatment with a topical GC is to actively reduce the risk for flares and extend the remission time, instead of only treating when lesions become clinically visible (14).

Oclacitinib

Oclacitinib is a Janus kinase (JAK) inhibitor. JAKs are nonreceptor tyrosine kinases that are activated by various cytokine receptors. In mammals, four JAK families (JAK1, JAK2, JAK3, and tyrosine kinase 2) exist, regulating the expression of multiple inflammatory genes. By selectively inhibiting JAK1- (and to a minimal extent JAK2-) dependent cytokines, oclacitinib can reduce the effects of pro-inflammatory and pro-allergic cytokines and is therefore considered as having a semi-broad working mechanism in CAD.

Because it has a fast onset of action for pruritus, oclacitinib is very useful for treating acute flares of itch. It is administered twice daily for 14 days and then continued with once-daily dosing (0.4-0.6 mg/kg). The twice-daily dosing is especially necessary when chronic dermatitis is present. It is considered safe for the long-term treatment of CAD in animals aged 12 months or more (15). Theoretically, oclacitinib may have immunosuppressive properties when used at dosages above recommendations (16), whilst its use in susceptible dogs may lead to opportunistic infections, viral papillomas, or demodicosis. In these cases the therapy should be discontinued, but in general routine hematologic evaluation, serum chemistry, and urine culture are not indicated for dogs receiving oclacitinib (17).

Figure 5. An atopic dog with iatrogenic Cushing's syndrome due to prednisolone treatment. Widespread firm calcinosis cutis lesions can be seen on the dorsum.

Cyclosporine and tacrolimus

Cyclosporine A is a calcineurin inhibitor with a specific immunosuppressive mode of action. It binds to the intracellular immunophilins, resulting in inhibition of the cytokine interleukin-2 (IL-2), leading to a reduction in T cell proliferation and antibody production by B cells dependent on T-helper cells. Cyclosporine also has a broad-spectrum working mechanism and should be dosed at 5 mg/kg q24H. However, efficacy onset is slow; it can take between 4-8 weeks before clinical signs of pruritus and dermatitis are in remission, so it can only be used for maintenance therapy in CAD. Concurrent treatment with other fast-acting drugs in the startup phase is found to be efficient and safe. Prednisolone given at 1 mg/kg q24H for a week, then continued by every other day dosing for two weeks, can be administered in the first three weeks of treatment with cyclosporine (18). Likewise, concomitant administration of oclacitinib (0.4-0.6 mg/kg q12H) for 14 days, then once daily for seven days, is well tolerated (17). Once an atopic dog is in remission with cyclosporine the dose should gradually be reduced (stepping down at around 1 mg/kg every two weeks) or given every other day to find the lowest possible dosing regime. Self-limiting side effects (*e.g.*, vomiting and diarrhea) occur in 30% of patients, predominantly within the first week of administration, so the author often uses a lower dose at startup (*e.g.*, 1.5 mg/kg q24H for 3 days, then 3 mg/kg q24H for another 3 days), especially in dogs with a sensitive digestive tract. Administration with food may also help to decrease gastrointestinal upset. Less frequent side effects, which may be dose-dependent, include gingival hyperplasia, excessive hair growth **(Figure 6)**, susceptibility for

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Figure 6. An atopic dog with excessive hair growth; the dog had received maintenance therapy with cyclosporine for a year **(a)**. The same dog as in **(a)** six months after switching to maintenance treatment with oclacitinib **(b)**.

opportunistic (fungal) infections, hyperplastic verrucous lesions, and psoriasiform-lichenoid-like dermatitis. However, these side effects usually regress if the drug is discontinued.

The calcineurin inhibitor tacrolimus has been found to reduce lesional scores when used topically for several weeks (19). Although tacrolimus can irritate the skin in the first days of treatment, a twice-daily application using a 0.1% preparation seems to be well tolerated in dogs.

Lokivetmab

The anti-canine interleukin-31 monoclonal antibody lokivetmab has a narrow mode of action and is the most specific symptomatic atopic therapy with the least side effects. It is capable of neutralizing canine IL-31, a cytokine involved in itching. Its mechanism of action differs from that of oclacitinib; IL-31 is bound by lokivetmab before it even can bind to its receptor, thereby preventing the main pruritogenic effects of the molecule. Monthly subcutaneous injections of lokivetmab can be used at 1-2 mg/kg (depending on national licensing data), with some dogs responding well to the higher dose, and remission of 4-8 weeks is commonly achieved. The drug has a very long half-life and can be safely used with other drugs for symptomatic CAD therapy. Its efficacy in reducing both pruritus and skin lesion scores (using the Pruritus Visual Analog Score (PVAS) and Canine Atopic Dermatitis Extent and Severity Index (CADESI) respectively) is shown to be non-inferior after 28 days of treatment to the broad-spectrum cyclosporine. While the initial response to lokivetmab is shown to be fast (reducing the PVAS by over 50% in 77% of atopic dogs), its overall efficacy after nine months of treatment was found to be 59% (20). The author's experience is that the drug has few or no side effects, and is excellent to treat dogs with mild to moderate pruritus, and notably some cases with insufficient response to oclacitinib may respond well to lokivetmab. However, it is less efficient for treating atopic dogs with severe (chronic) lesions, and although it is considered to be a fast-acting and safe product for CAD, the high cost may limit its use as maintenance therapy for many owners.

●●● Allergen-specific
●●● immunotherapy

Altering the immune response by allergen-specific immunotherapy (ASIT), also known as desensitization or hyposensitization, is the only disease-modifying therapy that neutralizes a hyper-responsive immune system to environmental allergens by inducing tolerance. ASIT can be defined as the practice of "gradually administering increasing quantities of an allergen extract to an allergic subject to ameliorate the signs associated with subsequent exposure to the causative allergen." Its mode of action includes the induction of allergen-specific regulatory T cells and their cytokine IL-10, induction of allergen-specific IgG4 levels, and reduction of both the ratio of Th2/Th1 cytokines and allergen-specific IgE levels (21).

Subcutaneous immunotherapy (SCIT) has been the mainstay of ASIT since the early 1980s. Two formulations are available for dogs, namely aqueous and alum-based solutions, and if the correct protocols are followed systemic side-effects are rarely seen. Adjustments to these protocols are often needed for the individual patient to improve efficacy or during certain periods (e.g., seasonal variation). For example, when a patient has flares one week prior to redosing, the injection interval should be shortened, or if a patient responds with increased itch after each injection, a lower dose may be needed.

In previous studies with atopic dogs, the overall success rate of SCIT was estimated to be 50-70% after 9 to 12 months of treatment (22). Attempts have been made to increase the efficacy and decrease onset time to clinical effectiveness by using a "rush" protocol, and studies in atopic dogs show this to be a safe and efficacious method (23). However, this approach is not currently recommended by the author, unless undertaken by specialists at referral centers.

An alternative to SCIT is sublingual immunotherapy (SLIT), whereby a specific allergen dose is administered orally once or twice daily. Owner compliance is essential for this, as the dog should not eat or drink for 10 minutes before and after the application. In the few uncontrolled open studies performed, no consistent evidence supporting the increased effectiveness of SLIT above SCIT has been shown (24).

Of particular interest is the recent novel intralymphatic mode (ILIT) of application, with recent studies reporting a quicker onset of clinical improvement and possibly a more sustainable efficacy over time (25).

Regardless of which ASIT mode of application is used, symptomatic therapy to control skin inflammation and pruritus should be given temporarily to maintain a good quality of life until the immunotherapy is judged to be effective. Because ASIT is a tailor-made treatment, it requires adjustments of dose, intervals, and control of flares to achieve the best results.

CONCLUSION

The atopic dog needs long-term multimodal treatment management to secure a good quality of life, and there is a need for education, clear explanation, and coaching of owners to achieve the best treatment outcome. Exacerbations of pruritus and dermatitis by secondary skin infections should be controlled with topical treatment, taking into account the repair of the epidermal barrier. In general, the more broad-spectrum a drug, the more side-effects can develop, and combinations of drugs such as glucocorticosteroids, cyclosporine and oclacitinib should be used sparingly due to the risk of enhanced immunosuppression when used long term. Allergen-specific immunotherapy is the only disease-modifying therapy for canine atopic dermatitis and should be tailor-made for the patient.

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CANINE ATOPIC DERMATITIS AND THE OWNER

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Dr. Prélaud graduated from Toulouse Vet School in 1984 and went on to establish a laboratory which pioneered canine allergy testing. A specialist in veterinary dermatology since 1987, he now holds a senior role at a private veterinary hospital outside Paris, and has also authored many articles and textbooks on canine atopic dermatitis.

Treating canine atopic dermatitis can be challenging in itself, but keeping the owner fully engaged can be challenging too, as Pascal Prélaud describes in this short paper.

KEY POINTS

Good and sustained communication with the owner is key to successful treatment of any dog with atopic dermatitis.

1

A clinic should have an established protocol to ensure an optimal approach to every atopic dermatitis case.

2

Introduction

Canine atopic dermatitis (CAD) is a multifactorial chronic disease that requires the veterinarian not only to deliver long-term care to the dog but also establish and maintain an excellent relationship with the owner. Unlike some other chronic diseases, there is no true consensus on the best monitoring program for CAD, but maintaining continued contact with these cases is essential to ensure the effectiveness, feasibility, and safety of the various treatment options, and this is only possible if the owner is fully informed and engaged. Optimal communication is therefore the cornerstone of long-term treatment, as CAD can negatively impact the owner's quality of life as well as that of the patient [1,2]. A recent large-scale survey on dogs with CAD (author's unpublished data, 2013) demonstrated both that owners frequently lack knowledge of the disease (for example, only 4% understood that CAD is a long-term disease), and that there are often shortcomings in the therapeutic options offered by the clinician (for example, only 15% had tried an elimination diet and only 6% had tried cyclosporine).

The first, most essential step with CAD is identifying the disease and getting owners to accept its lasting nature before starting on the long journey involved in monitoring a chronic skin condition. However, there are many potential pitfalls; one study suggested there are seven fundamental mistakes to avoid in long-term management of chronic dermatology cases **(Table 1)** [3]. This short paper provides some key measures that can be easily incorporated into daily practice to ensure that the owner of an atopic dog is kept fully engaged and better informed.

First steps: preparation

Atopic dermatitis is a real opportunity for veterinarians, in terms of medical, technical and interpersonal reasons, but it is essential to understand the disease in order to master the various therapeutic options and therefore communicate effectively. This involves the clinician keeping up to date with evidence-based journals and websites (*e.g.*, www.icada.org) [4], and having access to the appropriate equipment for dermatological investigations, including a good microscope and otoscope. Good "tools" to use during the consultation, such as checklists and even metaphorical diagrams [5-7], may be useful, and additional communication resources, such as informative websites, can also be helpful. Do note however that most canine atopic dermatitis sites

Table 1. Seven common mistakes when dealing with chronic skin conditions [3].

- Not controlling flare-ups
- Not taking client expectations into account
- Not understanding the situation in terms of quality of life
- Not using evidence-based medical data
- Underestimating the role of treatment compliance
- Not taking cost into consideration when offering appropriate therapeutic choices
- Regarding dermatology clients/patients as a nuisance

are either too technical or very product- or service-oriented, and it can be helpful for the clinic to have its own web page or blog designed specifically for owners of atopic dogs. In addition, follow-up consultations should always be assigned to the same veterinarian, and the clinic should have a trusted specialist for referral of difficult cases.

The consultation process

Certain elements are key to ensuring good client communication, and it is essential that a clinic has an appropriate protocol in place for dermatological cases **(Table 2)**. The first consultation should never be overlong, as this is generally unnecessary and counterproductive. A shorter timeframe is more effective, and should focus on a few vital points. The clinician should first fully understand any existing or previous therapies, and then address the owner's expectations and the limits of their motivation or adherence to treatment. It is also necessary to give an explanation (and if necessary a demonstration) of any medication prescribed, and – essentially – explain what it is intended to achieve. So, for example, if a dog with pre-existing CAD presents with a flare-up of otitis externa and *Malassezia* pododermatitis, the consultation should focus on the *Malassezia*, topical ear care, and the feasibility of treatment options. Prolonged explanations about allergies, immune responses or the skin barrier should be avoided, and keeps things simple; an appointment should then be made for a follow-up visit when other aspects can be discussed as necessary.

Subsequent consultations should be equally structured. To maintain a positive relationship with the client, and ensure case continuity, a telephone or video call within 48 hours of the initial consult can allow the clinician to check on treatment efficacy, adherence, and any adverse effects. A physical check-up 2 or 3 weeks later not only enables a second clinical examination (including aural and cytological checks), it allows further discussion on treatment and logical planning for future management. This can be aided by using a quality-of-life scale (8), whereby identification of the priority factors can greatly assist in making therapeutic choices **(Table 3)**. Information should essentially be provided in stages, with each consultation emphasizing only the necessary points for understanding the treatment.

Outside the consultation

As noted above, a quality-of-life scale is an essential support tool when carrying out a follow-up (6,8) and this can be completed online, during a video consultation, or even via a smartphone app. This is in preference to other options such as lesion charts or pruritus scales, which the author finds unhelpful. Keeping in contact is also essential, and for serious cases group meetings with owners may be beneficial. "Care contracts" which assist in monitoring chronic diseases are suitable for dogs with CAD, and financial planning with a monthly breakdown of expenses will keep the owner engaged and facilitate early detection of complications or flare-ups, which can help avoid losing contact with the case.

CONCLUSION

When dealing with CAD, longer consultations and over-reliance on external resources can be counterproductive, and it is much more effective if the veterinarian can show empathy for the owner's viewpoint; this must be coupled with a thorough understanding of canine atopic dermatitis and a willingness to offer appropriate treatment options, alongside judicious use of measures that help the owner comprehend the many ramifications of this complex disease.

Table 2. Key elements for good communication.

- Training for dedicated veterinarians and nurses
- Uniform approach within the clinic
- First consultation focusing on treating the flare-up
- Follow-up phone call within 48 hours
- Adaption of treatment(s) as necessary
- Simple, appropriate monitoring tools
- Follow-up scheduling

Table 3. A quality-of-life survey can help prioritize the factors to be considered when dealing with a dog with CAD (8). Scoring each factor (on a scale of 1 to 5) will identify the main criteria for both the dog and the owner.

Dog factors	Owner factors
• Disease severity • Behavior/mood • Sleep • Eating behavior • Work/play • Social relations • Behavioral changes • Treatments	• Loss of time • Exhaustion • Family activities • Cost • Emotional distress • Physical discomfort • Family relations

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A DIAGNOSTIC APPROACH TO CANINE OTITIS

Canine otitis is a frequent challenge for first opinion clinicians, and successful management is based on addressing the multiple causes and factors involved in its pathogenesis, as Hannah Lipscomb and Filippo De Bellis describe.

KEY POINTS

1 The pathogenesis of otitis externa involves primary and secondary causes and predisposing and perpetuating factors; understanding these causes and factors are all key to successful management.

2 When seeing a patient with otitis for the first time, a thorough history should always be taken, followed by physical and dermatological examination, otoscopy and ear swabs.

3 Routine microbiological culture of all otitis cases is not indicated, as it will not distinguish between resident commensal bacteria, overgrowth and infective organisms.

4 Imaging modalities available for assessing the ear include radiography, sonography, computed tomography and magnetic resonance imaging.

Introduction

Otitis is seen in first opinion veterinary practice on a regular basis (1), representing some 10-20% of all canine cases presenting to practitioners (2). Otitis externa (OE) (inflammation of the external ear canal, or EEC) is typically complicated by secondary infection which can – along with other factors – lead to rupture of the tympanic membrane (TM) and the development of otitis media (OM). Over 50% of dogs presenting with chronic OE have concurrent OM (3), and without intervention the cycle of aural inflammation and infection will continue, resulting in pain and irreversible pathological changes. Successful management is based on addressing the multiple causes and factors involved in the pathogenesis of otitis (4). The causes can be primary (*e.g.*, foreign bodies, ectoparasites, allergies, endocrinopathies, or immune-mediated diseases) or secondary (essentially infection from Gram-positive or Gram-negative bacteria and fungi) in nature, but other aspects are also important. These include predisposing factors (such as obstruction, conformation, aural environment, or topical treatment effects), and perpetuating factors (such as pathological changes resulting from chronic OE or OM). This article reviews the diagnostic approach to canine otitis and provides practitioners with a step-by-step guide to managing cases from first presentation.

Signalment and history

When first presented with a dog with either acute or chronic otitis it is important to be aware of the clinical history and to formulate a list of provisional primary causes. The consultation should start as normal, with thorough history taking to enable potential causes to be ruled in or out. To achieve this the following questions should be covered:

- **What is the dog's signalment?** Various studies have shown that Cocker Spaniels, Poodles, Pyrenean Shepherds and Labrador Retrievers are all predisposed to developing otitis due to the conformation of their pinnae, EECs and/or hereditary susceptibility (5). In young dogs, otitis can be caused by *Otodectes cynotis* – although this is less common with the newer oral and spot-on ectoparasiticides – whereas in old dogs an underlying endocrinopathy is more likely.

- **What are the owner's concerns?** They may describe head shaking, ear rubbing, aural discharge and malodor (6).

- **When was the complaint first noticed?** Abrupt and frenzied head shaking increases suspicion of an aural foreign body (6), whereas chronic cases are usually associated with clinical or subclinical disease.

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- **Is the otitis unilateral or bilateral?** Acute unilateral otitis increases the likelihood of an aural foreign body; chronic bilateral otitis is more likely to indicate other etiologies (*e.g.*, allergies) and can additionally be complicated by the ear anatomy.

- **What is the pet's lifestyle?** Does the dog exercise in fields or go swimming? Water trapped in the EEC changes the aural environment and can cause dysbiosis (6).

- **Does the dog suffer from seasonal flares of otitis?** If so, this is highly suggestive of primary allergic skin disease, such as non-food-induced atopic dermatitis.

- **Has any previous topical treatment been successful?** If not, this could indicate either a resistant infection or an adverse drug reaction.

Clinical examination

The next step is to perform a complete physical examination, followed by a specific dermatological examination. Clinicians typically have a routine for this, but generally it is advisable to start from the nose and work back towards the tail, thus ensuring all body systems are checked. When dealing with otitis, the physical examination may allow a tentative diagnose of OM, otitis interna (OI) or hypothyroidism. Clinical signs of OM include facial nerve paralysis (*e.g.*, head tilt, ear droop, lip droop and ptosis) and Horner's syndrome (*i.e.*, miosis, ptosis, enophthalmos and protrusion of the nictitating membrane). Clinical signs of OI include hearing loss and vestibular disease (*e.g.*, head tilt, asymmetric ataxia, leaning to the affected side, circling and horizontal nystagmus) (7,8). Hypothyroidism (other than the appearance of the skin and haircoat) is clinically associated with obesity, weakness, lethargy and bradycardia (9). However, any suspect diagnosis should be confirmed with appropriate investigation(s).

The dermatological examination should assess the skin in its entirety: periocular, perioral, dorsal and ventral neck, axillae, trunk (dorsum, ventrum and flanks), inguinal, perianal, interdigital (dorsal and palmar/plantar), pinnae and the EEC opening. Practitioners should be mindful of any skin lesions that could relate to otitis which may explain the primary etiology. For example, as well as signs of otitis, puppies with juvenile cellulitis may have erythema, oedema, exudation, crusting and alopecia of the face and muzzle (10), and dogs with atopic dermatitis may present with a classic combination of otitis, pododermatitis and superficial pyoderma.

When dealing with otitis, it is sensible to carefully examine the ears last, as they can be painful, and dogs may subsequently develop an aversion to their ears being touched. However, even with minimal handling it is possible to collect more information simply by examining the inner aspect of the pinnae and opening to the EEC: erythematous pinnae can suggest an allergic etiology, whilst chronic cases may have thickened, hyperpigmented pinnae with excessive scaling, which may represent a cornification disorder (6). Additionally, the appearance of any aural discharge can reveal primary or secondary causes for the otitis: a dry, brown, granular discharge is seen with *O. cynotis*, a moist, brown discharge commonly occurs with both staphylococcal and *Malassezia* infections **(Figure 1)**, and a purulent, malodourous discharge is typical of Gram-negative bacterial infection **(Figure 2)** (2).
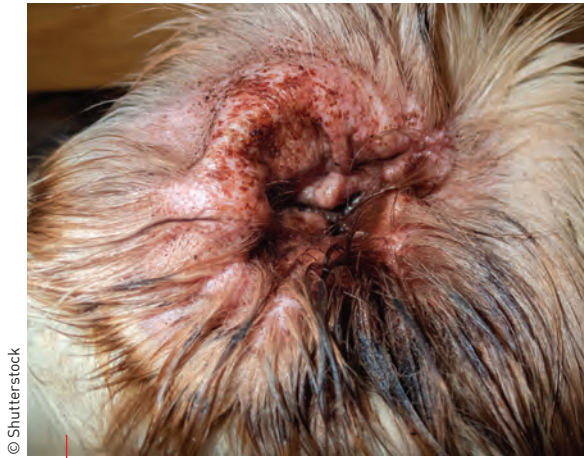
Otoscopy

If tolerated by the patient and once distant examination of the pinnae is complete, it is essential to perform otoscopy to evaluate the EEC and the integrity of the TM. There are three different types of otoscope available (11,12):

Figure 1. Moist, brown discharge on the concave aspect of the pinna, as seen with both staphylococcal and *Malassezia* infections.

**Figure 2.** Purulent discharge on the concave aspect of the pinna, typical of Gram-negative bacterial infection.

1. Closed otoscope – allows good visualization of the EEC and TM and is designed to allow air to be introduced into the canal in order to undertake tympanometry, although accessing the EEC via the scope (*e.g.*, to perform cytology) is limited.

2. Open otoscope – gives an inferior view of the EEC and TM compared to closed otoscopy but permits excellent access into the EEC. For this reason, all practices should have the option of an open otoscope.

3. Video otoscope – enables an excellent view and access to the EEC and TM, with the extra benefit of taking photographs and videos, although the equipment cost and the skill required for proper use can be an issue.

To fully appreciate the usefulness of otoscopy practitioners should be familiar with the appearance of the healthy aural anatomy. The normal EEC is a smooth, pale pink, thin-walled structure and the normal TM is a semi-transparent, concave membrane with a fine center and thicker periphery. The TM is divided anatomically into two: the dorsal section (*pars flaccida)* is light pink in color, whereas the ventral section (*pars tensa)* is pearl-grey **(Figure 3)**. For every patient and each ear an appropriately sized, sterile otoscope cone (stored at room temperature) should be used. The cone will gently slide along the intertragic incisure – the soft depression separating the tragus and antitragus cartilages at the base of the pinna – and into the EEC. Assuming this is tolerated by the patient, the vertical and horizontal portions of the canal can be examined; the junction between the two sections is distinguished by a prominent cartilaginous ridge, and the pinna should be elevated upwards and outwards to straighten the canal as best as possible (12). The otoscope cone can then be eased into the horizontal portion for improved visualization **(Figure 4)**. With experience it is possible to rapidly identify or assess for foreign bodies, *O. cynotis*, inflammation, exudate, stenosis, proliferation and TM status (11,12). As with all the diagnostic steps performed so far, otoscopy also contributes to the etiological understanding of otitis **(Table 1)** (11,12).

Otoscopy is commonly challenged by anatomy (*e.g.*, hairy ear canals), pathology (*e.g.*, excessive discharge and stenosis) and patient temperament. If problems are encountered, it is preferable to perform otoscopy under sedation or general anesthesia (GA) and, for stenosis, after a course of oral glucocorticoids (*e.g.*, 0.5-1.0 mg/kg prednisolone SID for 1-2 weeks, followed by appropriate tapering) (11,12). Otoscopy may detect an abnormal TM (*e.g.*, it may appear thickened, bulging, opaque and/or ruptured), but if its integrity is unclear it is possible to investigate this further under GA using either tube palpation or tympanometry. The first option involves using video otoscopy to pass a thin feeding tube or urinary catheter slowly along the EEC; in a healthy normal ear the tip of the tube or catheter will remain visible, but if the tip is lost from sight it has entered the middle ear. Tympanometry is a more skilled technique and is rarely practiced, as it involves gradually introducing air into the EEC via a closed otoscope; under normal circumstances the membrane will flex in a concave/convex manner in
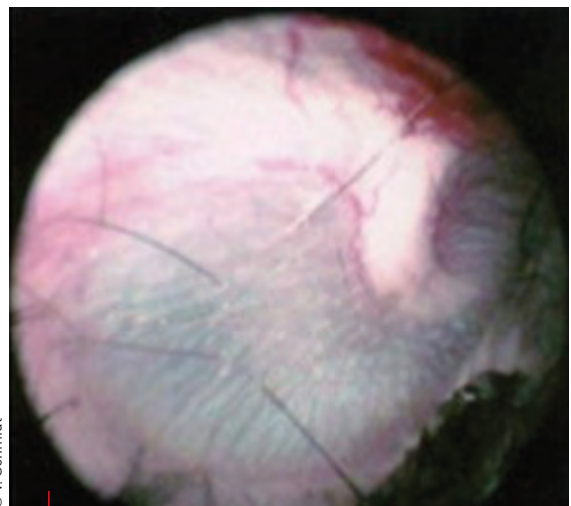
Figure 3. A video otoscope image of the normal tympanic membrane. Note the dorsal, light pink *pars flaccida* and ventral, pearl-grey *pars tensa*.

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Figure 4. Performing careful open otoscopy to allow visualization of the external ear canal and tympanic membrane in a conscious patient.

response to the air, but if it remains rigid or bulges, an accumulation of material in the middle ear is suspected (13).

Microscopic evaluation

After otoscopy it is essential to take a swab from the affected ear(s) for in-house cytology, and this should be performed for every patient. Obtaining a sample simply involves placing a cotton bud into the EEC for a few seconds, but the material in the horizontal canal is usually of most clinical relevance and safely swabbing this portion in conscious patients can be difficult. Therefore, advancing the bud until the cartilaginous ridge is reached, and swabbing at this junction, should be sufficient. The bud is then rolled onto a clean microscope slide and labelled (2). Separate slides are prepared for cytology and to check for ectoparasites (e.g., *O. cynotis* and *Demodex canis*), especially if the patient is a young dog. The ectoparasite slide should be prepared with a few

drops of mineral oil, the sample rolled on top and finished with a coverslip. Microscopic visualization for ectoparasites is maximized by using a low power (x4 or x10) objective and light intensity, and a closed condenser. The entire slide should be examined methodically with a "back and forth" or "up and down" pattern (2).

The slide for in-house cytology should be stained using a commercial modified Wright kit, consisting of a fixative and eosin and hematoxylin stains. The slide should be dipped for approximately 5 seconds, rinsed and dried. Starting on low microscopic power (x4 objective) and light intensity, and with an open condenser, a cellular area on the slide should be focused on. The power should then be increased to the highest power (x100 oil immersion objective), which will allow micro-organisms and inflammatory cells to be identified (2,14).

Under normal circumstances low numbers of bacteria (*e.g.*, coagulase-negative *Staphylococcus* spp., coagulase-positive *Staphylococcus* spp. and *Streptococcus* spp.) and yeasts (predominantly *Malassezia pachydermatis*) reside in the canine EEC. When the canal becomes insulted or inflamed, bacteria and/or yeasts can become opportunistic, overgrow and potentially cause infection. Studies have suggested mean micro-organism numbers per high power field (x40 objective) indicative of normal microflora versus an abnormally increased population; for bacteria this is 5 or less versus 25 or more, and for *Malassezia* it is 2 or less versus 5 or more **(Figure 5)**. Moreover, and in contrast to the normal aural flora, micro-organisms routinely contributing to otitis are coagulase-positive *Staphylococci*, β-hemolytic *streptococci*, *Pseudomonas* spp. and *Proteus* spp. (2,15).

Cytology also helps determine overgrowth from infection by the presence of inflammatory cells [predominantly degenerate or non-degenerate neutrophils **(Figure 6)**] but it is not possible to identify bacterial species on cytology – culture is required for this. Culture and sensitivity

Table 1. Otoscopic finding(s) and direct deduction.

Otoscopic finding(s)	Direct deduction
Erythematous and hyperplastic EEC	Acute otitis
Fibrotic and hard EEC	Chronic otitis
Erythema of the vertical ear canal with no discharge	Allergic otitis: primary etiology could be food-induced atopic dermatitis or non-food-induced atopic dermatitis
Erosions and ulcers of EEC with purulent discharge	Gram-negative bacterial infection
"Cobblestone" appearance of EEC lining	Sebaceous and ceruminous gland hyperplasia, capable of transitioning into polyp-like growths
Foreign bodies	Primary cause
Ectoparasites	Primary cause
Tumor	Predisposing factor

"Otoscopic examination may be challenged by the patient's anatomy, pathology and temperament; if encountered, it is preferable to perform otoscopy under sedation or general anesthesia and, where stenosis is present, after a course of oral glucocorticoids."
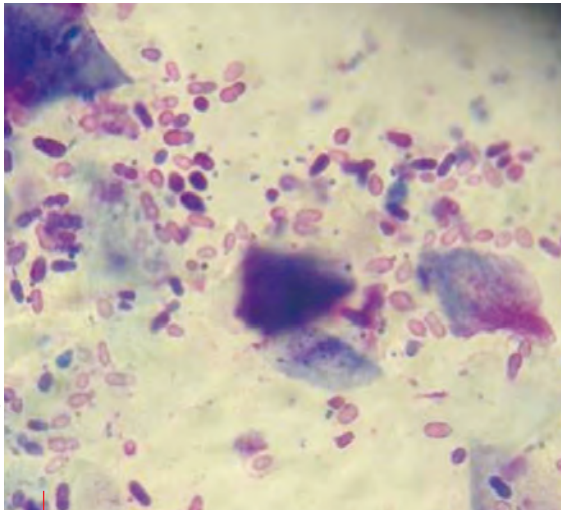
Hannah Lipscomb

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Figure 5. High number of *Malassezia* organisms from an infected ear are visible in this microscopic cytology image.
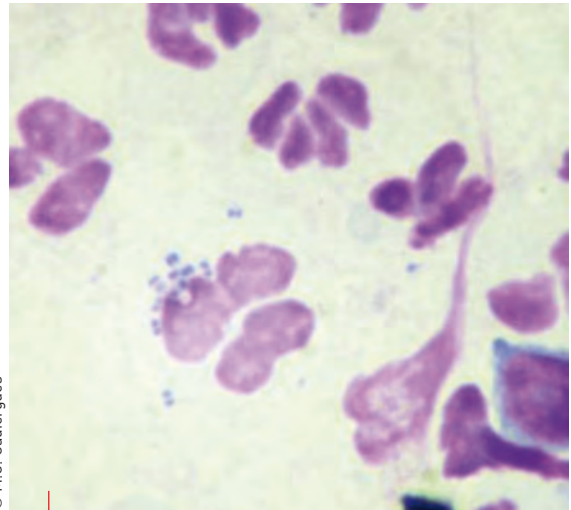

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Figure 6. A microscopic image showing non-degenerate and degenerate neutrophils and a cluster of extracellular cocci.

(C&S) testing should not be used routinely, but rather reserved for certain scenarios, as culture cannot distinguish between resident bacteria, overgrowth and infection, so the reported antibiotic sensitivity is for all micro-organisms present. The reporting of irrelevant bacteria can lead to inappropriate antibiotic therapy or unnecessary switching of treatment. Conversely, C&S may fail to culture important micro-organisms, misleading interpretation and causing a premature cessation of treatment (2). Culture is certainly indicated for chronic and medically unresponsive cases of OE, when rod-shaped bacteria are identified on cytology, or if OM is present. Additionally, studies have proven that different micro-organisms can independently cause infections in the external and middle ear; patients with OE and OM should therefore have samples taken from both areas, which could potentially produce two sets of results with differing antibiotic sensitivity patterns (16).

Diagnostic imaging

Imaging can allow further assessment of an otitis case, and especially the status of the middle ear. The literature recommends imaging for cases of suspected OM, para-aural abscessation, trauma, nasopharyngeal polyps, neurological dysfunction, and if a dog is unable to open its mouth (17). Moreover, imaging can help determine the direction of treatment: medical versus surgical. Ear canals that are associated with bony and irreversible pathological changes are more likely to be treated surgically (18).

- Radiography of the skull to assess the EEC and middle ear should be done under GA; this should consist of left and right oblique views, a dorsoventral skull view and an open-mouth rostrocaudal view; the latter is the best option to evaluate the tympanic bulla (TB). Imaging may confirm occlusion and bony changes of the EEC, content within the TB, and lysis or proliferation of the TB wall. However, the pathology must be severe to be detectable, and subtle changes

are easily missed (17,19). Radiography can also be used to assess the TM integrity using a technique called positive contrast canalography. This requires a soluble non-ionic iodine contrast medium to be introduced into the EEC and allowed to diffuse by gravity for a few minutes before obtaining dorsoventral and rostrocaudal open mouth radiographs. If the TM is ruptured, contrast medium may be identified in the middle ear; however, if a patient has stenotic ear canals the contrast may not reach as far as the middle ear, even if the TM is not intact. Consequently, caution must be used when interpreting the radiograph when using this procedure (20).

- Sonography allows the TB to be assessed, with the probe placed on the ventrolateral surface of each bulla; tiny movements of the probe allow the bullae to be scanned for fluid or a mass lesion. The major disadvantage of this modality is that a high level of skill is needed (17,19,21).
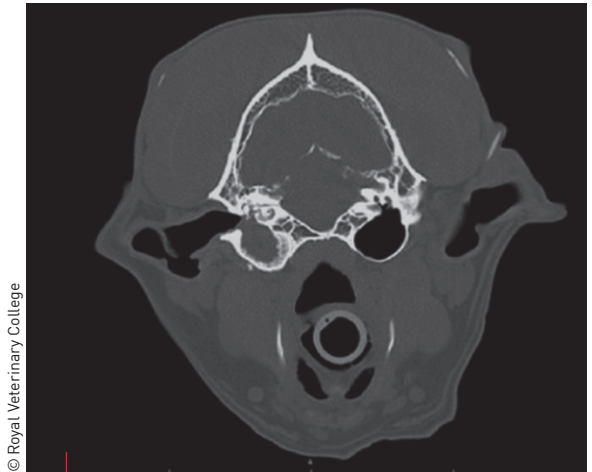

"Cytology should be performed for every patient after otoscopy, and obtaining a sample is quick and easy: however, the material in the horizontal canal is usually of most clinical relevance and safely swabbing this section in conscious patients can be difficult."

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- Computed tomography (CT) and magnetic resonance imaging (MRI) are advanced imaging modalities which can be useful with some otitis cases. CT allows excellent visualization of the bony structures local to the ear, and is excellent for diagnosing stenosis or occlusion of the EEC and filling of the TB **(Figure 7)**. MRI offers the best resolution for soft tissue structures, and is preferable if masses within or around the ear are suspected, although it is less sensitive at highlighting the cartilage of the EEC and the TB (17,19).

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Figure 7. A transverse CT scan of a dog's skull showing soft tissue or fluid within the right tympanic bulla with thickening of the bulla wall.

Myringotomy

The TM will be intact in approximately 70% of OM cases, as the middle ear can become infected without OE by micro-organisms migrating from the pharynx via the auditory (Eustachian) tube or by hematogenous spread. Cavalier King Charles Spaniels and brachycephalic breeds can also have a primary OM with no EEC pathology (22). In cases when OM is diagnosed but the TM is intact, myringotomy (iatrogenic rupture of the TM) is required. This is performed under GA guided by video otoscopy after the EEC has been thoroughly lavaged and allowed to dry. With direct visualization a 6F urinary catheter, cut obliquely at 60° and attached to a 2 mL syringe, is advanced through the most ventral part (6-7 o'clock) of the TM. One milliliter of sterile saline is infused into the middle ear and aspirated; the sample is transferred to a sterile tube and centrifuged to prepare samples for in-house cytology and C&S testing. If the middle ear subsequently requires further treatment the puncture site can be carefully enlarged for easier access and repeatedly lavaged until clean and empty (7,11).

CONCLUSION

Practitioners should follow a step-by-step approach when dealing with canine otitis to avoid misunderstanding the case, as this will inevitably lead to treatment failure. It is important to consider both primary and secondary causes, as well as predisposing and perpetuating factors, and a logical progression will elicit useful information that helps confirm the status of both the external canal and the middle ear. In short, the more thorough the investigation, the more likely is long-term treatment success.
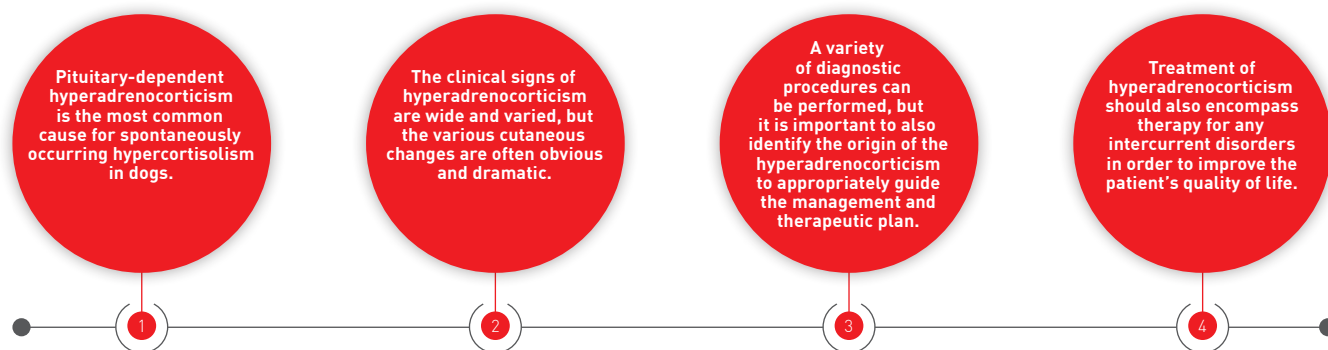
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CANINE HYPERADRENOCORTICISM

Dogs with hyperadrenocorticism can often present with dermatological signs; this paper reviews the diagnosis and treatment of a common canine disease.

KEY POINTS

1 Pituitary-dependent hyperadrenocorticism is the most common cause for spontaneously occurring hypercortisolism in dogs.

2 The clinical signs of hyperadrenocorticism are wide and varied, but the various cutaneous changes are often obvious and dramatic.

3 A variety of diagnostic procedures can be performed, but it is important to also identify the origin of the hyperadrenocorticism to appropriately guide the management and therapeutic plan.

4 Treatment of hyperadrenocorticism should also encompass therapy for any intercurrent disorders in order to improve the patient's quality of life.

Introduction

Canine hyperadrenocortisolism is a relatively common condition which may occur either spontaneously or via an iatrogenic route. The spontaneous etiologies include hypersecretion of endogenous glucocorticoids from a functional adrenal tumor or hypersecretion of corticotropin or corticotropin-like substances from an idiopathic functional pituitary tumor, whilst exogenous glucocorticoid administration can lead to iatrogenic disease. Approximately 85% of dogs with spontaneous hypercortisolism have pituitary-dependent hyperadrenocorticism (PDH), which results from excessive corticotropin secretion arising from either a microadenoma or a macroadenoma of the pituitary gland (1). Approximately 90% of all pituitary tumors are functional, with the hypersecretion of corticotropin resulting in bilateral adrenal hyperplasia.

The hypothalamic-pituitary-adrenal axis

The adrenal cortex is composed of three distinct anatomical regions, the zona glomerulosa, zona fasiculata and zona reticularis, with glucocorticoids being produced in the zona fasiculata under the control of the hypothalamic-pituitary-adrenal (HPA) axis. The hormone corticotropin (or adrenocorticotropic hormone, ACTH) is secreted by the adenohypophysis of the pituitary gland, with the primary function of stimulating the adrenal cortex. Secretion occurs in a pulsatile manner and is stimulated by stress, but is normally controlled by the negative feedback of serum glucocorticoid levels. Corticotropin in turn is controlled by release of corticotrophin releasing hormone (CRH), secreted by the hypothalamus, again in a pulsatile manner (2,3). CRH secretion is inhibited by glucocorticoids and stimulated by serotonin and epinephrine.

Diagnosis

Since diagnosis of canine hyperadrenocorticism can be complex and none of the screening tests are 100% accurate, an integrated approach to the diagnosis is required. Signalment, history, clinical findings, screening tests and specific assays for the hypophyseal-adrenal axis should all be considered carefully in a collective manner to avoid misdiagnosis and to ensure concomitant disorders are not overlooked.

Signalment, history and clinical signs

Hyperadrenocorticism typically affects middle-aged to old, small breed dogs, with no apparent gender bias. Whilst any breed can develop the disease, Poodles, Dachshunds and Terriers appear to be at increased risk. The clinical signs are generally slow to develop and progress, and many owners consider the early stages as part of the normal aging process of their dog. Various cutaneous changes, as shown in **Table 1**, are often significant. These include the classic textbook appearance of generalized bilateral symmetrical

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truncal alopecia **(Figure 1)**, often accompanied by hyperpigmentation **(Figure 2)**. Thinning of the skin **(Figure 3)** and calcinosis cutis are also commonly seen **(Figure 4)** and suppression of the immune system can contribute to chronic dermatitis and furunculosis **(Figure 5)**. Other systemic signs are also commonly encountered, as set out in **Table 2** (4). It is important to question an owner about recent corticosteroid administration (topical, oral, and injectable) to rule out a possible iatrogenic cause for hyperadrenocorticism before attempting to diagnose the spontaneous form of the disease.

General health profile findings

If a dog is suspected of having hyperadrenocorticism after collecting the signalment, history and performing a physical examination, then blood and urine sampling (hematology and biochemistry panels, plus urinalysis and culture) is indicated. Routine laboratory findings in dogs with hyperadrenocorticism are shown in **Table 3**.

Diagnostic tests

A presumptive diagnosis of hyperadrenocorticism is often made from the clinical signs, physical examination and routine laboratory findings, but diagnosis should be confirmed by a hormonal assay (5-7). Various tests are available to assess the HPA axis.

Low-dose dexamethasone suppression test (LDDST). Many clinicians consider the LDDST as the diagnostic test of choice for canine hyperadrenocorticism, largely because it has a 90-95% sensitivity for dogs with PDH (8). However, the specificity can be low, therefore in animals suspected of having non-adrenal disease it is best to wait until the dog has recovered from the concurrent illness prior to testing for hyperadrenocorticism. To perform the

Table 1. Cutaneous signs of hyperadrenocorticism.

- Bilateral, symmetrical hypotrichosis/alopecia
- Coat color change
- Hyperpigmentation
- Thin, hypotonic skin
- Comedones
- Calcinosis cutis
- Poor wound healing
- Phlebectasia (venous dilation)
- Bruising (petechiae and ecchymoses)
- Seborrheic dermatitis
- Suppressed immune function (Chronic recurrent superficial pyoderma, *Malassezia* dermatitis, demodicosis, dermatophytosis)

Figure 1. Owners may believe that some of the signs that can develop with hyperadrenocorticism, such as bilateral trunk hypotrichosis (one of the most common signs of the disease), are a normal part of aging. Unusually, in this dog some hair regrowth is visible along the Blaschko lines.

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Figure 2. Generalised bilateral symmetrical alopecia of the trunk with consequent intense hyperpigmentation of the skin due to exposure to UV light.

Figure 4. Calcinosis cutis (white spots) and comedone formation (black spots) which can be typical of Cushing's disease.

Figure 3. The abdomen of a dog with Cushings disease. Note the thin skin with superficial blood vessels easily visualized, and an area of hypocollagenosis where the skin appears to be torn.

Figure 5. A dog with severe furunculosis on the right hind limb; this was due to ruptured inflamed hair follicles with free hair shafts in the dermis leading to a foreign body reaction at the affected areas.

test dexamethasone sodium phosphate is administered at 0.01 mg/kg intravenously, and serum cortisol concentrations are determined at 0, 4 and 8 hours afterwards. If the dexamethasone fails to adequately suppress circulating cortisol concentrations at both 4 and 8 hours (with levels remaining > 1 μg/dL or > 30 nmol/L) then hyperadrenocorticism can be confirmed in a dog with compatible clinical signs, although this does not determine the underlying cause. However, if there is initially suppression of cortisol levels at 4 hours post-dexamethasone (levels < 1 μg/dL or < 30 nmol/L) followed by a demonstrable increase in cortisol by 8 hours, this identifies PDH as the cause of the hyperadrenocorticism, with approximately 30% of dogs with PDH showing this "escape pattern" (5-7,9).

Corticotropin (ACTH) stimulation test. This is the best screening method to distinguish between dogs with iatrogenic and spontaneous hyperadrenocorticism. As well as being convenient and quick to perform, it also provides useful baseline information for treatment monitoring when using mitotane or trilostane (5-7). The preferred method is to take a baseline sample for serum cortisol before intravenous or intramuscular injection of cosyntropin (ACTH) at 5 μg/kg, and then determine serum cortisol an hour later. Affected dogs tend to have an exaggerated response to the cosyntropin administration, with cortisol concentrations rising to greater than 20 μg/dL (> 600 nmol/L). Low-normal baseline results with little to no response to ACTH stimulation is diagnostic for iatrogenic hyperadrenocorticism. This test will identify about 85% of dogs with PDH (5-8,10,11), but will not discriminate between PDH and an adrenal tumor if hyperadrenocorticism is present, therefore additional diagnostics, such as an abdominal ultrasound, are required. It is important to note that stressed dogs, or those with significant non-adrenal illness, can have cortisol levels elevated enough to give false positive results with this test. Ideally, a dog should be allowed to recover from its nonadrenal illness before testing is performed.

Urine cortisol: creatinine ratio. A non-specific screening test that has a high sensitivity (85-99%) and exceptionally low specificity, the

Table 2. Systemic signs of hyperadrenocorticism.

- Polyuria/polydipsia
- Abdominal distension
- Polyphagia
- Panting
- Weakness and lethargy
- Muscle atrophy
- Neuromuscular signs (pituitary macroadenomas may cause seizures, circling or blindness)
- Reproductive abnormalities (persistent anestrous, testicular hypoplasia)
- Recurrent urinary tract infections
- Diabetes mellitus
- Acute pancreatitis

urine cortisol: creatinine ratio (UCCR) is used for its negative-predictive value and is really only useful in ruling out hyperadrenocorticism.

Once a diagnosis of hyperadrenocorticism is confirmed it is then important to determine whether the patient has a functional adrenocortical tumor or PDH. Endocrine tests which can help the clinician to differentiate the etiology include the high-dose dexamethasone suppression test and plasma endogenous ACTH concentration. Imaging techniques such as abdominal radiography, abdominal ultrasound or computed tomography/ magnetic resonance imaging can also be particularly helpful **(Table 4)**.

High-dose dexamethasone suppression test (HDDST). This test may be used when Cushing's disease has been confirmed by means of a LDDST in order to clarify whether a dog has the pituitary or the adrenal form of the disease; a HDDST will identify the cause of the hyperadrenocorticism in approximately 75% of affected dogs. The protocol is identical to the LDDST, but with the dexamethasone administered at 0.1mg/kg IV. If the cortisol levels are suppressed, the diagnosis is PDH.

"Since the diagnosis of canine hyperadrenocorticism can be complex and none of the screening tests are 100% accurate, an integrated approach is required, with signalment, history, clinical findings, and screening tests all taken into account."

Fiona Scholz

Table 3. Routine laboratory findings in dogs with hyperadrenocorticism.

Hematology
- Stress leukogram (neutrophilia, lymphopenia and eosinopenia) - Erythrocytosis
Serum biochemistry panel
- Elevated alkaline phosphatase (ALKP)* - Elevated alanine transferase (ALT) - Hypercholesterolemia - Hyperlipidemia - Hyperglycemia - Low blood urea nitrogen (BUN)
Urinalysis and urine culture
- USG: hyposthenuric (often < 1.008) providing drinking water has not been withheld - Glucosuria (if concurrent diabetes mellitus) - Possible bacteruria and proteinuria, often without pyuria

*85-90% of dogs with hyperadrenocorticism exhibit elevated ALKP (5-7)

Plasma Endogenous ACTH concentration. Endogenous ACTH concentrations are normal to high in dogs with PDH (> 40 pg/mL or > 8.8 pmol/L) and low (< 20 pg/mL or < 4.4 pmol/L) in those with adrenal tumors. Unfortunately, about 20% of dogs with hyperadrenocorticism will have non-diagnostic results in the "grey" zone, therefore diagnostic imaging or HDDST is required to determine the cause of the hyperadrenocorticism (4). Furthermore, sample handling can be both difficult and costly, so this test is not routinely used and the clinician should discuss sample collection and processing requirements with a local laboratory before proceeding.

Table 4. Diagnostic imaging techniques to support PDH diagnosis.

Imaging technique	Comments
Radiography	Not useful in confirming PDH, but the presence of mineralization in the region of the adrenal gland may suggest an adrenal tumor. Absence of mineralization does not rule it out.
Abdominal ultrasound	Particularly useful in distinguishing dogs with PDH from those with adrenal hyperadrenocorticism. Bilateral adrenal gland hyperplasia > 7.5 mm is consistent with PDH in a dog with confirmed hyperadrenocorticism. Sonography should only be used to determine the cause once a diagnosis has been established using the pituitary function tests outlined in the text.
Computed Tomography (CT) or Magnetic Resonance Imaging (MRI)	With either technique bilateral adrenal enlargement can be readily differentiated from a unilateral adrenal tumor. Both techniques are useful to confirm pituitary tumors, MRI is more accurate at visualizing small pituitary tumors as it facilitates superior soft tissue contrast (12).

●●●● Management

Before embarking on treatment, all concurrent disorders such as urinary tract infections and diabetes should be identified and treated. Although these may not completely resolve until the hypercortisolism is controlled, they can become life-threatening to the patient if ignored. Treatment of any demodicosis or secondary bacterial or *Malassezia* skin infections is also important, as resolution of these will improve the patient's quality of life.

Calcinosis cutis **(Figure 6)** usually resolves with removal of the underlying cause, but frequent bathing in medicated shampoos and hydrotherapy are helpful. Occasionally, the surgical removal of isolated lesions may be recommended if the surgeon feels wound healing would be successful in an individual patient. Calcinosis cutis can also be treated with dimethyl sulfoxide (DMSO) gel once or twice daily until resolved (13). Serum calcium levels should be monitored, as calcium release from the larger nidus in the tissue may elevate levels. More recently the use of minocycline to treat calcinosis cutis has been reported (14). Although an antibiotic, minocycline chelates calcium and directly inhibits collagenolytic enzymes, but it is important to remember that resolution is not immediate and that the skin often looks worse before it looks better.

Trilostane

Trilostane acts by inhibiting cortisol steroidogenesis, as it is a competitive inhibitor of the 3-β-hydroxysteroid dehydrogenase enzyme system. The induction dose is 2-5 mg/kg PO daily (usually divided into two) and is generally well tolerated, although reported adverse effects include lethargy, decreased appetite, anorexia and vomiting. Hypoadrenocorticism

"The clinical signs of hyperadrenocorticism are generally slow to develop and progress, and many owners consider the early signs as part of the normal aging process of their dog; the various cutaneous changes are often significant."

Sam Crothers

© Christoph Klinger

Figure 6. Calcinosis cutis with surrounding post-inflammatory pigment alterations. The calcinosis usually resolves with removal of the underlying cause, but frequent bathing and hydrotherapy may also help.

can occur with overdosage but should resolve rapidly following discontinuation of the drug. The most serious potential side effect is acute adrenal necrosis, and although deaths are rare, subclinical histopathological evidence of cortical necrosis is more common. The underlying cause of the necrosis is uncertain and cannot be directly explained by the competitive inhibition of steroidogenesis; it may be due to hypersecretion of ACTH, which, as well as increasing the size of the adrenal glands, may also paradoxically result in necrosis and hemorrhage of the tissue.

Mitotane (o,p'-DDD)

Mitotane can be used because it causes selective necrosis of the zona fasciculata and reticularis of the adrenal cortex, whereas the zona glomerulosa (which produces mineralocorticoids) is relatively resistant (13). The induction dose (administered with food) is 12.5-25 mg/kg q12H for 7 to 10 days (15). The most common side effects seen initially include signs of hypoadrenocorticism, including lethargy, vomiting, diarrhea, anorexia and weakness (16). If such signs occur, therapy should be discontinued, and glucocorticoids administered. Less commonly, disorientation, ataxia, head pressing and acute hepatopathy may be seen (17).

Water consumption or appetite may be measured to provide response to treatment, with the latter being a more precise way to monitor mitotane therapy in many cases. The dog is fed 75-80% of its normal ration, and the owner instructed to observe the point at which the dog fails to finish a meal. If water consumption is used, the owner should be alert for this decreasing to < 60 mL/kg/day. When a

reduction in water or food consumption is noted, or after 7-10 days of mitotane therapy, another ACTH response test should be performed to determine whether cortisol suppression is adequate. If this is the case, cortisol levels should be in the normal range both before and after ACTH administration. To maintain suppression of cortisol secretion, mitotane is then administered at a dosage of 50 mg/kg per week. Dogs on long-term treatment should be examined and have an ACTH response test performed every 3-4 months, as incremental doses are often required to maintain adequate clinical remission.

Other options

Ketoconazole has a reversible inhibitory effect on glucocorticoid synthesis whilst having minimal effects on mineralocorticoid production, and has been used effectively to manage canine hyperadrenocorticism, although around 33-50% of all dogs treated will fail to respond adequately. The initial recommended dosage is 10 mg/kg q12H for 14 days, although treatment can be initiated at 5 mg/kg q12H for the first seven days to assess drug tolerance, before increasing to 10 mg/kg. The efficacy of the initial 14-day course of treatment is determined by an ACTH stimulation test.

Selegiline (L-deprenyl) hydrochloride is an irreversible monoamine oxidase (type B) inhibitor that increases dopamine levels, which in turn can inhibit ACTH release from the pituitary gland. Treatment is initiated at 1 mg/kg daily, but increased to 2 mg/kg if the response is inadequate after two months. However, only 10-15% of dogs show improvement of clinical signs with this treatment (3).

Radiation therapy of pituitary tumors is associated with a high rate of response, although most dogs require trilostane or mitotane therapy for several months after radiation treatment because of residual ACTH secretion.

Hypophysectomy has been performed successfully in dogs with PDH, but the surgery is technically difficult and not widely available. Thyroid and glucocorticoid support may be needed after surgery, and animals may lose the ability to secrete vasopressin, leading to diabetes insipidus.

CONCLUSION

Early recognition of the clinical signs of hyperadrenocorticism should allow the necessary diagnostic tests to be initiated and appropriate therapy commenced if the disease is confirmed. Dogs should be checked 6-8 weeks after starting treatment, at which point there should be a marked improvement, with the most obvious and rapid response being a reduction in water intake, urine output and appetite. The skin and hair coat changes can take longer to resolve – sometimes several months – and the dermatological signs may deteriorate markedly before improving. Re-examination every 3-6 months is recommended for the remainder of the animal's life, as relapses and episodes of overdosage can occur, and regular assessment of adrenal reserve by ACTH stimulation testing is indicated.
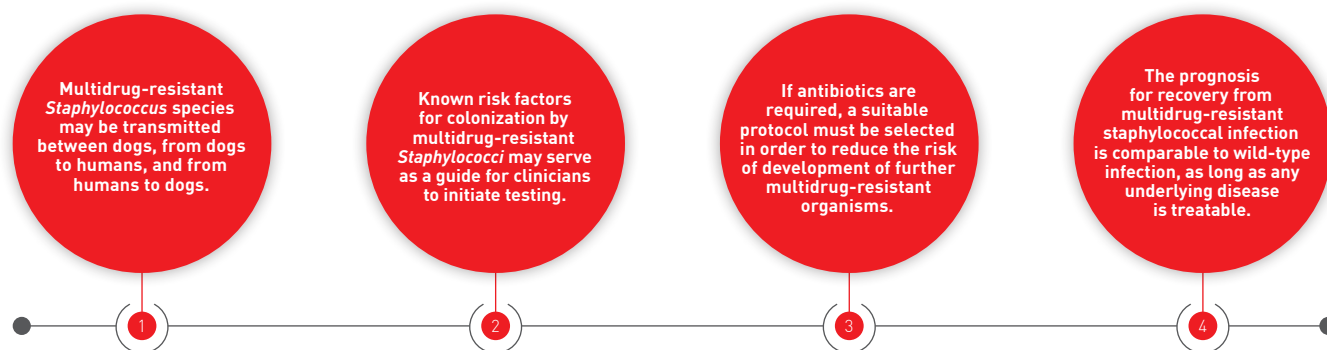
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MULTIDRUG-RESISTANT STAPHYLOCOCCAL SKIN INFECTIONS

Management of multidrug-resistant staphylococcal infections poses a considerable challenge to veterinary practices, but the problem is not insurmountable with the right protocols in place, as this article describes.

KEY POINTS

1 Multidrug-resistant *Staphylococcus* species may be transmitted between dogs, from dogs to humans, and from humans to dogs.

2 Known risk factors for colonization by multidrug-resistant *Staphylococci* may serve as a guide for clinicians to initiate testing.

3 If antibiotics are required, a suitable protocol must be selected in order to reduce the risk of development of further multidrug-resistant organisms.

4 The prognosis for recovery from multidrug-resistant staphylococcal infection is comparable to wild-type infection, as long as any underlying disease is treatable.

Introduction

Infections with multidrug-resistant *Staphylococci* (MDRS) are commonly encountered in both human and veterinary medicine, and such infections are challenging on both an individual case basis and a community level. The prevention of colonization and infection with MDRS is important in maintaining the health of patients, veterinary staff and the public, and there have been numerous publications concerning the risk factors for development of, testing for, and treatment of MDRS in recent years. This article provides a practical overview of MDRS infection in dogs, including how and when to test, the implications for the home and veterinary practice environments, and management strategies to both resolve infection and prevent re-infection.

What's the background of *Staphylococcus* species?

Staphylococcus is a genus of Gram-positive, coccoid bacteria that can be classified into several groups. In the veterinary field the most significant groups are coagulase-positive *S. intermedius* (*S. pseudintermedius*, *S. delphini* and *S. intermedius*) and *S. aureus* (1).

S. pseudintermedius is the most common bacteria isolated from healthy dogs, with the highest carriage rates (in descending order) on the oral mucosa, perianal skin, nasal mucosa and groin (1), and it has been shown that dogs with atopic dermatitis have a higher colonization rate compared to their healthy counterparts (2). *S. aureus* is a commensal of the skin and nasopharynx of healthy humans and, as with *S. pseudintermedius*, can also be an opportunistic pathogen (3).

Colonization and subsequent infection by *Staphylococci* occur via bacterial adhesion to corneocytes, but this is variable. It is known that *S. pseudintermedius* adheres with greater affinity to canine over human corneocytes (1), whereas *S. aureus* has a lower affinity for canine compared to human corneocytes, and canine nasal carriage of methicillin-resistant *S. aureus* (MRSA) is thought to resolve rapidly without treatment (4). Transmission of *S. pseudintermedius* from dogs to humans is possible but uncommon. Following adhesion to corneocytes, indirect transmission of both susceptible and MDRS species may occur via shedding of desquamated cells into the environment, and it is therefore important to implement infection control whether there is active infection or simply colonization with MDRS.

How is multidrug resistance defined?

Multidrug resistance (MDR) is not a term exclusive to *Staphylococci*, as it defines any bacteria showing resistance to one or more antibiotics in at least three

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different classes; for example, *S. pseudintermedius* showing resistance to cephalexin, clindamycin and doxycycline, or *Pseudomonas aeruginosa* showing resistance to marbofloxacin (or enrofloxacin), gentamicin and polymyxin B (5). Methicillin-resistant *Staphylococci* (MRS) defines a genetically distinct group of *Staphylococci* with resistance to β-lactam antibiotics. The resistance is due to acquisition of the mecA gene that encodes for penicillin-binding protein (PBP2a), a transpeptidase involved in bacterial cell wall synthesis. PBP2a has a lower affinity for β-lactam antibiotics than other transpeptidases (6), and the mecA gene confers resistance to most β-lactam antibiotics including methicillin, penicillin and the majority of cephalosporins. With MRSA, progression to multidrug resistance occurs with the accumulation of multiple resistance genes around the mecA gene inside the bacterial "cassette" (the SCCmec) (7).

In humans there are two main routes of infection by MRSA: hospital associated, and community acquired. The hospital infections are nosocomial (*i.e.*, acquired whilst the patient is hospitalized or undergoes a medical procedure) whilst the latter occur in patients with no healthcare contact, and have distinct pheno- and genotypes distinguishing them from hospital-acquired MRSA (8). In dogs, cutaneous infections with MRSA are much less common than infections with methicillin-resistant *S. pseudintermedius* (MRSP) (9).

How is MDR identified and treated?

Whenever a MDR infection is suspected, certain steps must be taken to protect the health and welfare of the patient, clients, staff and other animals that may directly or indirectly contact the bacterium. Once the presence of infection is confirmed via cytology, MDR is determined via culture and susceptibility testing (CST). Where the MDR infection is a *Staphylococcus* species the carriage status, via sampling of staphylococcal carriage sites, should be established. Effective infection control measures are then implemented in order to reduce shedding of MDRS into the home and veterinary practice environments, and to reduce the risk of transfer to other animals and humans. Finally, appropriate treatment that is effective in resolving the infection but avoids further selection for antimicrobial resistance must be chosen.

How is infection confirmed?

The first step in the investigation of any suspected cutaneous bacterial infection is the identification of consistent lesions on physical examination **(Figure 1)**, followed by skin sampling and cytology. It is important to note that *Staphylococci* cultured from a non-sterile site (such as the skin or ear canal) does not
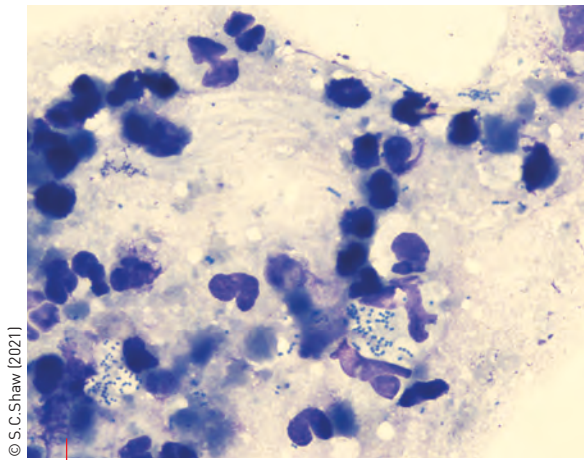

Figure 1. Multiple papules, pustules and epidermal collarettes on the ventral abdomen of a dog with atopic dermatitis, consistent with superficial bacterial pyoderma.
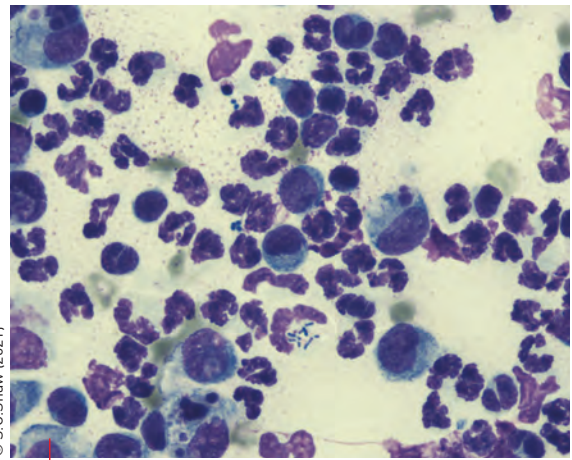
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Figure 2. x100 oil immersion of a direct impression smear showing multiple neutrophils with intracellular cocci bacteria (consistent with bacterial infection).

Figure 3. x100 oil immersion of a direct impression smear showing pyogranulomatous inflammation with intracellular bacilli and cocci bacteria (consistent with bacterial infection).

confirm the presence of infection, only the presence of the bacterium at the test site. Bacterial infection is confirmed via cytology which demonstrates the phagocytosis of bacteria by neutrophils and/or macrophages following appropriate sampling of a skin lesion **(Figures 2 and 3)**.

How is testing for MDRS performed?

Once bacterial infection is confirmed by cytology, CST can be performed to determine the species of bacteria involved and establish susceptibility to systemic antimicrobial drugs. Note that routine susceptibility panels do not provide information on susceptibility to topical antimicrobials. CST should be performed in all cases where systemic antimicrobials are deemed necessary for treatment. The morphology of the phagocytosed bacteria observed on cytology should match that of the cultured bacterium to verify that the bacteria causing the infection is the same as the one cultured.

As with conventional bacterial infections, MDRS may be identified by aseptic sampling using a sterile bacteriology swab. The sample is submitted in transport media suitable for aerobic bacteria (*e.g.*, Amies Bacterial Transport Medium) with or without charcoal for routine CST. PCR testing for mecA is the gold standard for identification of methicillin resistance (10), but this is not always available at commercial laboratories, and so diagnosis is usually based on selective culture. CST can be used to confirm the presence of MDRS in one of two situations; either at the site of infection and/or at staphylococcal carriage sites (*i.e.,* assessment for carriage status).

1. For any suspected case of MDRS, CST should be performed at the site of infection. In bacterial infections where topical antimicrobial treatment is likely to be adequate (*i.e.,* the majority of bacterial skin and ear infections), culture determines the presence of MDRS to inform appropriate infection control. Recognizing the

risk factors for the development of MDRS/MRS can prompt the clinician to perform testing **(Table 1)**. One of the main risk factors for MRSP colonization is prior exposure to antibiotics, so CST should be considered for any patient presenting with a bacterial infection that has recently received a course of antibiotics for any reason. Numerous classes of antibiotics have been shown to select for MRS, and MRSP may be isolated from carriage sites even after MRS pyoderma has resolved (11). Empirical use of antimicrobials should therefore be avoided unless there is a risk to life, or if delaying treatment will result in significant morbidity.

2. In animals with confirmed MDRS infection, it is prudent to assess for colonization of carriage sites by MDRS. Colonization in itself does not dictate the need for decontamination, but knowledge of its presence is important for infection control; a colonized dog has the potential to shed and transmit the bacterium from sites other than the site of infection. For this type of MDRS testing, cytology is not required, as only the presence of MDRS is needed to confirm colonization. Three bacteriology swabs from the nasal mucosa, gingival mucosa and perianal skin may be

Table 1. Risk factors for MRS* (12,13).

Patient factors	Environmental factors
• Chronic dermatological disorders	• In-contact people or animals with skin disease
• Infections unresponsive to empirical antibiotic therapy	• Individuals in a household working in a healthcare environment
• Patients previously diagnosed with MRS	• Household members who have had MRS in the past, including other pets
• Patients treated with multiple courses of antibiotics	
• Non-healing wounds	
• Recently hospitalized patients	* MRS: Methicillin-resistant *Staphilococci*
• Frequent veterinary visits	

submitted as a pooled sample for culture. Positive results indicate MDRS to be present at one or more of the carriage sites. As active decolonization is not necessary in the majority of cases, susceptibility testing of carriage sites is not required, although where susceptibility testing of carriage sites is performed, around 80% of *Staphylococcus* spp. isolated are the same as in pustules from remote body locations (14). Some dogs, particularly those living in environments where strict hygiene protocols are not followed, can carry MRSP for more than 12 months (15).

What about carrier animals?

Carrier animals are individuals whose staphylococcal carriage sites (nasal and oral mucosa, perianal skin) are colonized with MDRS in the absence of active infection elsewhere on the body. Dogs may be long-term carriers of MRSP, but only temporarily (days to weeks) carry and shed MRSA. In humans, testing to identify asymptomatic MRSA carriers is not done, but those at risk of infection (*e.g.*, individuals preparing to undergo surgery) are tested and decolonized as appropriate. Decolonization also takes place if there are high-risk people in the same household or recurrent infections in a household member (16). In the veterinary field a proactive testing system for patients at risk of MDRS infection should be adopted, and, as with the human situation, those scheduled for complicated procedures (particularly surgery for permanent implants) should be tested and decolonized as necessary.

Routine testing for carrier status in patients that have recovered from MDRS infection is sometimes advocated, and as noted above, dogs may shed MRSP for up to a year following resolution of infection. In countries where the prevalence of MRSP is low, use of environmental control measures and antimicrobial treatment of carriage sites until two consecutive negative carriage site screens (a reasonable time between tests is 3 weeks) are obtained, is a sensible method to reduce shedding of MRSP (16).

Infection control in the clinic

A number of studies have identified veterinary staff to be at higher risk for carriage of both MRSA and MRSP compared to the general population (17). It is therefore essential that practice protocols are in place to prevent transfer of and infection with MDRS in staff and patients. Simple measures may be employed to reduce both direct transmission of MDRS between patients and staff and indirectly via fomites. Personnel can actively reduce the spread of bacteria by washing their hands in an approved manner with soap and water, or where hand washing is not possible, use of an alcohol-based hand sanitizer (16).

Cleaning and disinfection are required to reduce environmental contamination with MDRS. Amongst the disinfectants commonly used in veterinary practice, those containing quaternary ammonium and hydrogen peroxide have been shown to kill *Staphylococcus* spp. (16). It is important to remove all organic matter from surfaces by routine cleaning prior to use of disinfectants, as they cannot percolate through organic debris and biofilms, and MDRS may be able to survive in these microenvironments.

The following protocol may be used to reduce the risk of direct transmission and environmental contamination of MDRS when dealing with outpatients with active infection and/or MDRS carriage:

- The patient should be seen at the end of the day, and should wait outside the practice until the consultation.
- Infected wounds should be covered prior to entry into the practice.
- The patient should be taken straight into the consultation room, avoiding the waiting area if at all possible.
- Trolleys should be used to transport patients if possible (to reduce the potential for contamination of the floor before it can be cleaned and disinfected).
- The consult room should be clean and contain only equipment necessary for the patient being seen.
- The room and trolley (if used) should be cleaned and disinfected immediately following the consultation.

"**Multidrug-resistant** *Staphylococci* **species (MDRS) are commonly encountered in both human and veterinary medicine, and provide a challenge on both an individual case basis and community level... prevention of colonization and infection with MDRS is important in maintaining the health of patients, veterinary staff and the public.**"

Eleanor K. Wyatt

Appropriate personal protective equipment (PPE) should be used by all staff in direct contact with the patient. This consists of gloves, apron/gown/overalls, sleeve protectors (if a bare-below-the-elbow policy is not already in place) and shoe covers. A change of clothes following contact with MDRS patients is required unless the staff member is completely covered by PPE. Clothing should be placed in a bag during transport and washed at 60°C for 10 minutes and tumble dried if possible (18). Masks are not necessary to prevent respiratory infection, as the bacteria are not airborne, but they may be useful in preventing staff from touching their faces, and therefore reducing the risk of colonization with MDRS (16).

For MDRS patients requiring hospitalization, the following steps may be taken to minimize the risk of transmission of MDRS to staff and other patients and contamination of the environment:

- The patient's MDRS infection site(s) should be covered with an impermeable dressing.
- Patients should be housed in an isolation ward.
- The number of staff interacting with the patient should be kept to a minimum and PPE should be worn (as above).
- If the patient needs to be moved, this should be done via a trolley to avoid contamination of floors.
- Gloves should be changed following removal of dressings from infected wounds, prior to application of clean dressings.

Infection control in the home

Home management of MDRS patients poses several challenges, as both the pet and the environment are potential reservoirs of infection for in-contact people and other animals. The home is also generally less amenable to the cleaning and disinfection protocols used in a medical environment. However, management of MDRS infection and natural decolonization is possible in the home and is generally preferable to hospitalization, as it reduces the risk of transfer to a larger number of people and potentially high-risk patients.

The risk of infection with MRSP in healthy people is low, but immunosuppressed individuals and those with open or surgical wounds are at greater danger of infection; they should be provided with specific advice on how to reduce the risk and advised to consult with their doctor for further support. Contact between the patient, its environment and any high-risk individuals should be prevented wherever possible. Where this is not practical, prevention of direct contact should be attempted by housing the animal and high-risk person in different areas of the home. Steps that may be taken to minimize transmission in the home include:

- Wash bedding and toys daily – such items have been associated with infection and carriage of MDRS in pets (16).

- Apply alcohol hand gel and/or wash hands following contact with the pet.
- Prevent the pet from licking people.
- If exercising other dogs in the household, keep them on leads and avoid areas where other animals are likely to be encountered, such as parks.
- Prompt disposal of feces followed by hand washing.
- Gloves (and other PPE) should be used when handling the infection site.
- Frequent cleaning and disinfection of the pet's environment – consider isolating the pet to an area of the house that is easy to clean.
- Prevent the pet from sleeping in the owner's bed.

Treatment options for MDRS infection

Management of infection can be challenging due to the reduced number of antimicrobial treatment options and the need for strict infection control protocols. Despite this, the prognosis for recovery from MDRS against wild type infection is the same, provided any underlying condition predisposing to infection (*e.g.*, atopic dermatitis) is treatable (16). The choice of antimicrobial agent depends on the infection severity (surface, superficial or deep) and extent (localized or widespread) **(Table 2)**. Topical antimicrobial therapy should be considered for all cutaneous bacterial infections due to the higher local concentrations achieved compared with systemic antimicrobials.

Topical therapy

As with any superficial bacterial skin or ear infection, first-line therapy for MDRS infection is topical antimicrobials – for example, 2-4% chlorhexidine, which is effective against MDRS *in vivo* (19). One study demonstrated marked improvement of clinical signs in seven out of ten dogs with superficial pyoderma following 2-3 times weekly bathing in a 3% chlorohexidine-based shampoo for 10 minutes over 21 days (20).

Table 2. Choice of antimicrobial agent.

Surface pyoderma	Topical
Superficial pyoderma	Topical Widespread infections may require systemic
Deep pyoderma	Topical Systemic required in most cases
Wounds	Topical May require systemic for surgical wounds
Otitis externa and uncomplicated otitis media	Topical (must not be ototoxic if otitis media present) Systemic therapy for otitis interna

This regime would be appropriate for superficial pyoderma involving MDRS. Topical chlorhexidine is also commercially available in wipe, foam/mousse and spray formulations; daily use of such products can be an adjunct to bathing and may help speed resolution of infection, and some owners also find them easier to use.

Another topical antiseptic shown to be effective against MDRS is sodium hypochlorite (NaOCl), the active ingredient in bleach. A 6.15% solution of sodium hypochlorite has been shown *in vitro* to have bactericidal activity against MRSP at dilutions between 1:32 and 1:265 (21). Non-perfumed dilute household bleach may be used as a rinse following routine shampooing once to twice weekly, for example 5 mL of 5% bleach in 2 liters of waters to make the rinse. NaOCl is the sodium salt of hypochlorus acid (HOCl), an oxidizing agent widely used as a disinfectant and commercially available as a spray and hydrogel for treatment of skin infections in animals. HOCl has been shown to be effective against MRSP, extended spectrum β-lactamase-producing *Escherichia coli* and MDR *P. aeruginosa* in an *in vitro* pilot study (22).

Serious adverse effects from topical therapy are uncommon and limited to acute hypersensitivity reactions and contact dermatitis. However, regular use of chlorhexidine shampoo and/or NaOCl rinses may cause excessive drying of the skin, necessitating use of a moisturizing shampoo or conditioning spray.

Systemic therapy

For deep bacterial pyoderma or infections that are unlikely to respond to topical therapy alone (*e.g.*, widespread superficial bacterial pyoderma in an immunosuppressed animal), systemic antimicrobials are usually indicated. Such therapy applies selection pressure on both the infection-causing bacteria and the skin and gut microbiota. For this reason, selection of the narrowest spectrum antimicrobial, used for the minimum time period to resolve the bacterial infection, is recommended in order to reduce the risk of development and shedding of further MDR organisms. Where systemic antimicrobials are deemed necessary, drug choice should always be guided by susceptibility testing, with topical antimicrobials used adjunctively to speed resolution of infection and reduce the need for systemic drugs. If appropriate, topical antimicrobials may still be considered for first line therapy and should be instituted whilst susceptibility results are pending. In addition to antimicrobials, anti-inflammatory drugs may be helpful in some cases in resolving cutaneous infections, particularly those affecting the ear canal. In cases where infection has resulted from cutaneous inflammation and/or there is severe inflammation present because of chronic disease, short-term anti-inflammatory doses of systemic and/or topical corticosteroids can be of benefit in immunocompetent animals.

> "Effective infection control measures to reduce shedding of MDRS into the environment, and to reduce the risk of transfer to other animals and humans, must be implemented, alongside appropriate treatment that is both effective in resolving the infection and not further selective for antimicrobial resistance."
>
> Laura M. Buckley

The evidence for the length of treatment required for MDRS infections is currently limited. Repeat CST can be performed at the carriage sites at the end of a treatment course to monitor response, and this should be performed no sooner than at seven-day intervals. The current guidelines for duration of treatment for superficial pyoderma is three weeks, or one week beyond clinical resolution, and four to six weeks, or two weeks beyond clinical resolution, for deep pyoderma (16).

What about biofilm management?

One of the many defense mechanisms of *Staphylococci* is the ability to produce biofilm. This can severely hinder treatment of MDRS infections, especially those involving skin folds, the ear canals and surgical implants. Biofilms are a community of *Staphylococci* producing and growing within a protective extra-cellular matrix, which serves as a physical barrier between the bacteria and both systemic and topical antimicrobial agents. In the human field there have been a number of initiatives to try to combat biofilms, including removal of implants and infected foreign bodies, and high dose topical and systemic antimicrobials. Physical removal of the biofilm by washing, wiping or flushing is a crucial step in resolving the infection. There are also several novel treatments currently undergoing investigation, including metal chelators such as ethylenediaminetetraacetate (EDTA), enzymes, phytochemicals and bacteriophages, but further studies are required (23). Topical N-acetylcystine (NAC) is used to disrupt biofilm in

both veterinary and human medicine; available as a solution in combination with tris-EDTA, it may be used to flush the skin and ears to break down biofilm prior to use of antimicrobial agents. Both NAC and tris-EDTA have been shown to be effective anti-*S. pseudintermedius* and *P. aeruginosa* biofilm agents *in vitro* (24).

CONCLUSION

Long-term management and prevention of MDRS infection requires identification and management of any primary disease process predisposing to bacterial infection. Evidence-based guidance on the use of antimicrobial agents for the prevention of MDRS infection is lacking, but systemic antimicrobials can encourage the development of MDRS infection and should be avoided unless absolutely necessary. Whilst resistance to topical antimicrobial agents is also possible, regular use of such treatments may be useful in prevention of bacterial overgrowth and infection in susceptible animals. Long-term treatment success is inextricably linked with the underlying cause of the infection, and if it can be identified and successfully managed the prognosis is generally good. Failure to address any underlying disease makes relapse of MDRS infection more likely.

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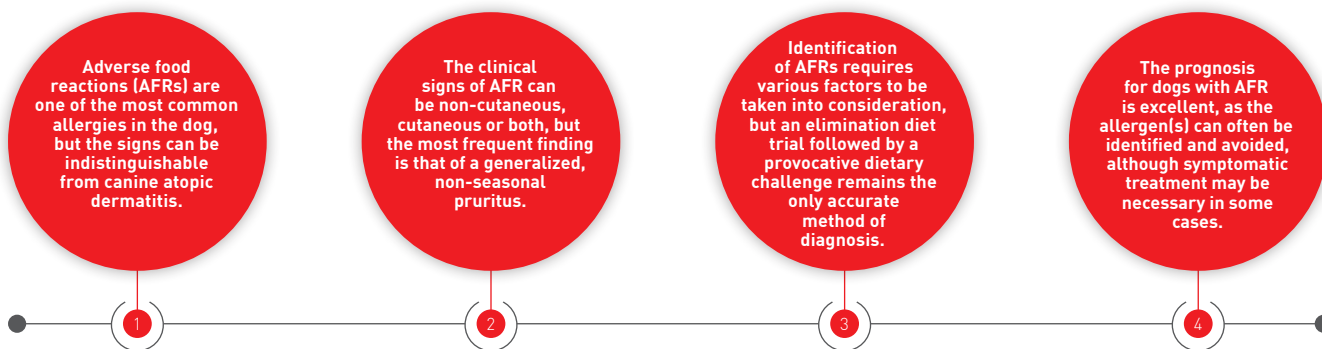
AN OVERVIEW OF ADVERSE FOOD REACTIONS IN DOGS

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Adverse food reactions can mimic many other skin disorders, and a good knowledge of the underlying pathology and diagnostic options are key to successful treatment of the condition.

KEY POINTS

1 Adverse food reactions (AFRs) are one of the most common allergies in the dog, but the signs can be indistinguishable from canine atopic dermatitis.

2 The clinical signs of AFR can be non-cutaneous, cutaneous or both, but the most frequent finding is that of a generalized, non-seasonal pruritus.

3 Identification of AFRs requires various factors to be taken into consideration, but an elimination diet trial followed by a provocative dietary challenge remains the only accurate method of diagnosis.

4 The prognosis for dogs with AFR is excellent, as the allergen(s) can often be identified and avoided, although symptomatic treatment may be necessary in some cases.

Introduction

The term "adverse food reaction" (AFR) refers to any abnormal clinical reaction resulting from the ingestion of food or food additives, and can be categorized as either toxic or non-toxic in nature (1,2). The first type is caused by substances that are natural food components, or that are present after food preparation or contamination; they can occur in any individual and are dose-dependent. Non-toxic adverse food reactions, in contrast, depend on the susceptibility of the individual, and are classified as either food intolerances (*i.e.*, non-immune-mediated) or food allergies (*i.e.*, immune-mediated) **(Figure 1)**.

Food intolerances, which (at least in humans) account for most AFRs, include enzymatic reactions and those resulting from the pharmacological properties of food (1,3). Food allergies are abnormal immunological responses to ingested food, and are specific and reproducible (4). In humans these responses may be IgE mediated, non-IgE mediated or mixed. IgE-mediated responses are the most studied (and best defined in literature), and include urticaria and angioedema, rhinoconjunctivitis, laryngeal edema, dysphonia, oral allergic syndrome, gastrointestinal signs, systemic anaphylaxis and exercise-induced anaphylaxis (5). The group of non-IgE-mediated disorders includes dermatitis herpetiformis, enterocolitic syndrome, colitis, proctitis, gastroesophageal reflux, celiac disease and pulmonary hemosiderosis. The mixed hypersensitivity category includes atopic dermatitis, esophageal and gastrointestinal eosinophilic disorders, and asthma. In dogs it is more difficult to make this differentiation, both because there are insufficient studies on the pathogenetic mechanisms of AFR and because clinical manifestations are not as heterogeneous as in humans, and the clinical picture often overlaps.
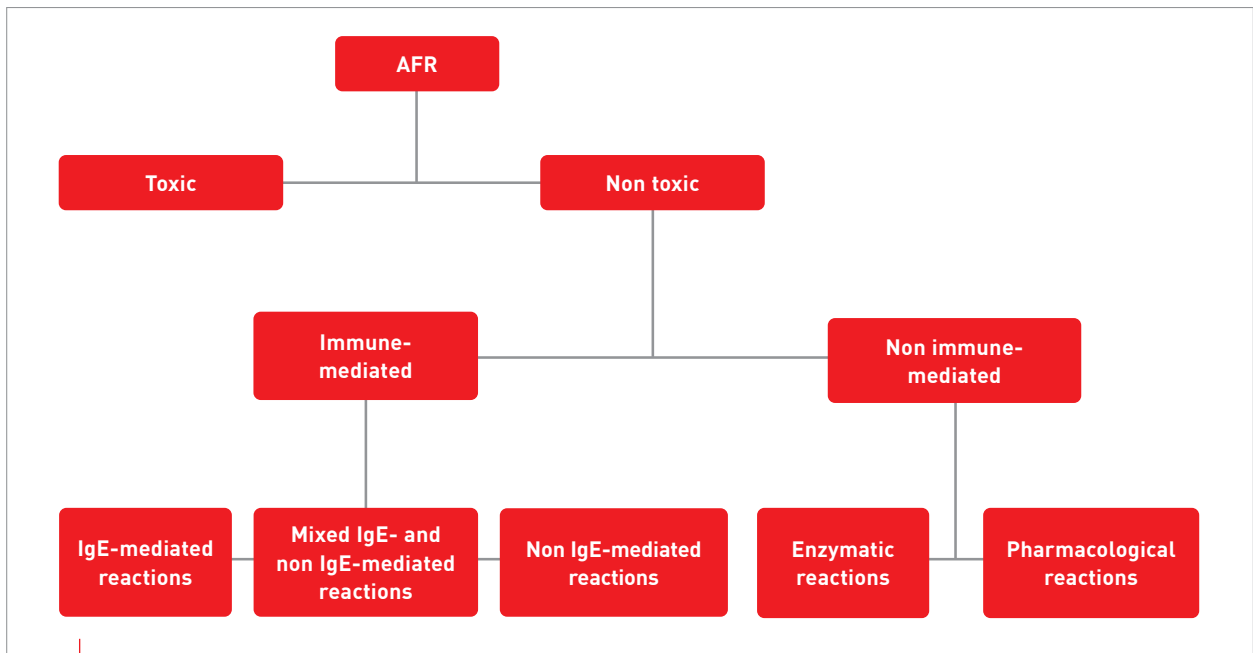
Figure 1. Classification of adverse food reactions.

In addition, there is no accurate test for their diagnosis and differentiation, so the more general term "adverse food reactions" is used to refer to this group of canine allergies.

Incidence, prevalence and predispositions

AFR is the third most common canine skin allergy (after flea bite hypersensitivity (FBH) and canine atopic dermatitis (CAD)). It is estimated that approximately 25-30% of dogs on a dietary elimination trial show a response to the altered diet and thus have an adverse reaction to food. A recent systematic review reported that the prevalence of AFR varied depending upon the type of diagnosis made: 1-2% of any diagnosis; 0-24% among skin diseases; 9-40% of dogs with pruritus; 8-62% of dogs with any skin allergic condition; and 9-50% of dogs with skin lesions suggestive of atopic dermatitis (6). However, diagnosis of an adverse skin reaction to food is only confirmed by a relapse of signs after provocation with the food responsible for the reaction. Not all studies involve provocation tests, so AFR may be over-diagnosed, as many animals can respond because the new diet is of higher quality, or because of other therapeutic interventions (*e.g.*, antiparasitic, antimicrobial or shampoo treatments) given in conjunction with the diet.

Pathology and possible triggers

The pathogenetic mechanisms of AFR are not fully understood. The gastrointestinal tract is continuously exposed to foreign antigens from food, microbiota or pathogens, and while some of these antigens are harmless, others are dangerous and must be removed. A breach in the mucosal barrier promotes local inflammation and increases the interaction between the luminal antigen and mucosal immune system.

In a healthy animal, lymphocytic activation occurs only when a potentially dangerous allergen comes into contact with the immune system. Conversely, when an external but non-hazardous allergen (such as a food allergen) is captured, various mechanisms are put in place to induce tolerance. The process that inhibits lymphocytic activation is called oral tolerance, and it is now recognized that there are multiple mechanisms involved, with one of the prime determinants being the dose of antigen fed. Low doses favor the induction of regulatory T cell (Tregs), whereas higher doses favor the induction of anergy or deletion, although these processes are not exclusive and might have overlapping functionality.

Although these mechanisms are very efficient in the majority of the population, individuals may be sensitized against food because of a deficient induction of oral tolerance or a breakdown in established oral tolerance (7). As yet it is not fully understood why these abnormal responses occur, but it is clear that the cause is multifactorial: both host and food-related factors are involved (8).

Signalment

A recent study analyzing signalment data from 825 dogs with food allergy produced useful information. The age at onset varied from a few months to 13 years, with an average of 2.9 years (9). 22% of dogs showed the initial clinical signs within the first 6 months of age and 38% when less than a year old. The most represented breeds were the German Shepherd (13%), the West Highland White Terrier (WHWT) (11%), and Labrador and Golden Retrievers (19%), which together comprise more

than 40% of all cases. Labradors and WHWT were considered to be predisposed when compared to the prevalence of these breeds in the normal population. There is no definite trend for sex predisposition, which seems to vary widely between studies, with a median female/male ratio of 0.9.

Clinical appearance

Adverse reactions to food can be difficult to diagnose due to the lack of pathognomonic signs. Non-seasonal pruritus is the most common clinical sign and often the first to appear. Itching is mainly localized in the ventral area, in particular the axillae, groin, and paws (on the palmar and/or plantar surfaces and dorsal interdigital areas). Itching of the ears is also frequently noted. A recent critical review that evaluated the dermatological signs of canine AFR suggested that approximately 50% of affected dogs demonstrate generalized pruritus **(Figure 2)** and that anal irritation, although reported in some individuals, is uncommon (4-25%) (10).

Although itching often occurs in typical areas, it is not pathognomonic, as many other skin diseases will involve the same regions, particularly other forms of hypersensitivity such as non-food-induced atopic dermatitis and FBH. Erythema and papules, with a distribution similar to that of the pruritus, are often reported as an adverse reaction to food **(Figure 3)**, whilst other skin signs may include self-trauma caused by the dog scratching or licking itself, brownish discoloration of the hair on the paws **(Figure 4)**, hypotrichosis, alopecia, excoriations and crusts. Over time, skin trauma causes hyperpigmentation and lichenification, and can lead to secondary skin infections **(Figures 5 and 6)**. If not treated promptly, bacteria and/or yeasts perpetuate the inflammation **(Figure 7)**, aggravating the dog and setting up a vicious cycle whereby the pruritic sensation leads to increased scratching and a worsening of the self-trauma.

Between 13-100% of AFR cases can resemble CAD (*i.e.*, an inflammatory, itchy skin with characteristic clinical signs), but it can also present as recurrent

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Figure 3. Ventral erythema and papules in a dog with AFR.

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Figure 4. A brownish discoloration of the hair on the paws, caused by dried saliva, can be indicative of AFR.

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Figure 2. A dog with generalized pruritus and secondary, self-induced skin lesions.

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Figure 5. Chronic mild to moderate lesions (erythema and hyperpigmentation) in a dog with AFR.

Figure 6. Chronic severe lesions (hyperpigmentation, lichenification and alopecia) in a dog with AFR.

Figure 7. Secondary bacterial infection in a dog with AFR.

Figure 8. Ceruminous otitis externa.

much rarer, enteropathies linked to AFR have also been reported, and are characterized by chronic intermittent or persistent diarrhea with a notable response to elimination diets.

Finally, AFR can be associated with conjunctivitis, and (rarely) respiratory disease – including bronchitis, rhinitis and chronic obstructive pulmonary disease – and even convulsions.

Diagnostic findings

The diagnosis of AFR is based on history, clinical signs, exclusion of other pruritic diseases and a dietary trial **(Figure 9)**. Because the signs are various and non-pathognomonic, other differentials (parasitic, infectious and allergic causes) must be considered. Ectoparasitic infestations (*e.g.*, *Sarcoptes* mange) and FBH can be excluded by skin testing and ectoparasite control. Secondary bacteria and yeast infestations should first be confirmed cytologically and then appropriately treated. If signs are still present after these causes have been excluded, then an allergic etiology is likely. However, it is necessary to differentiate between AFR and CAD, since the clinical signs can be identical and there are no laboratory tests that allow a reliable differentiation.

AFR is typically diagnosed following an elimination diet trial. This involves administering a foodstuff based on either a protein source novel to the dog's immune system, or a diet based on hydrolyzed protein. Note however that commercial diets can vary in the degree of protein hydrolyzation, and the clinician should select the diet with care (13). Some authors recommend the use of home-made recipes rather than commercial "hypoallergenic" diets because this decreases the risk of mistakenly introducing unwanted food components, but these can be problematic – for example, they can be nutritionally unbalanced, time-consuming to prepare, and expensive, especially for large breeds.

Commercial hypoallergenic diets should employ an extensively hydrolyzed protein source; although they may contain protein sources commonly eaten by the dog (*e.g.*, chicken), an effective processing method will remove the allergenic epitopes, which prevents the immune system from recognizing the culprit allergen.

superficial pyoderma (in 11-70% of cases). External otitis is commonly seen (3-69%) and is often associated with pruritus (80%) but may also be the only symptom (11,12) **(Figure 8)**. Other possible presentations include pyotraumatic dermatitis (1-9%), or – less frequently – *Malassezia* dermatitis, urticaria and perianal fistulae. Angioedema, urticarial vasculitis, neutrophilic leukocytoclastic vasculitis, oral allergy syndrome, erythema multiforme and interdigital furunculosis secondary to AFR have all been reported rarely.

In addition to dermatological signs, AFR may also cause gastrointestinal signs; these include chronic diarrhea and/or vomiting, soft fecal consistency or increased frequency of defecation. Abdominal pain, borborygmi and flatulence are also reported. Concurrent gastrointestinal and dermatological signs have been observed in 6-44% of affected dogs, but are not considered pathognomonic. Other,

An eight-week elimination diet trial should allow diagnosis of 90% of AFR cases (14) although a recent study showed that a shorter period is possible if the pruritus and inflammation are controlled with glucocorticoids during the first 2 weeks of the trial; dogs that do not relapse after glucocorticoid discontinuation can be provocatively challenged earlier, reducing the total time period for diagnosis (15).

Dogs that respond to the restricted diet should then be challenged by either their previous diet or its individual ingredients (at least 7-14 days for each food component), to assess for any recurrence of clinical signs. Note that individual animals can be allergic to several proteins, with 40% of dogs reacting to two ingredients and 20% to three or more (16). Only dogs that improve when given the restricted diet and then show an exacerbation of signs once re-exposed to the offending allergen(s) are definitely diagnosed as having AFR.

Control and management

There is no cure for AFR and strict avoidance of food allergens is the only way to prevent relapses. However, accidental exposures are not uncommon, and although relapses are not life-threatening, they are unpleasant and can diminish the quality of life for both dogs and their owners, and short-term intervention may be required. This can involve topical glucocorticoids, which are beneficial for localized lesions, or systemic treatment when the lesions or pruritus are generalized. The author's preference is for either oclacitinib (0.4-0.6 mg/kg q12H PO as long as necessary to control the relapse, then discontinued) or prednisone or methylprednisolone (0.5-1.0 mg/kg PO per day either once or twice daily) (17-19), with the dose gradually tapered to withdrawal once remission is achieved. The latter option tends to give a more rapid improvement than cyclosporine.

When culprit allergens cannot be identified or when accidental exposures are too frequent, long-term safer therapies are to be recommended. This typically involves oral oclacitinib or cyclosporine, as

"Adverse reactions to food can be difficult to diagnose due to the lack of pathognomonic signs. Non-seasonal itching is the most common clinical sign and often the first to appear."

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History
Signalment
Lesions: type, onset, distribution
Seasonality
Infectivity
Treatment response

↓

Clinical examination
General examination
Dermatological examination

↓

Exclusion of parasitic and infectious pruritic differentials
Regular ectoparasitic treatment
Treatment of bacterial and yeast secondary infections

↓

If clinical signs are still present: selection of an elimination diet
Home-made
Commercial with new proteins, carbohydrates and lipid sources
Commercial hydrolyzed

↓

Elimination diet for at least 8 weeks

↓

No improvement
AFR is unlikely

Improvement < 50%
Extend the elimination diet for 2-4 weeks

Improvement > 50%

↓

Improvement < 50%
Consider concurrent allergies (CAD, FBH) and/or secondary infections

Improvement > 50%
AFR is possible

↓

Perform the provocative test

↓

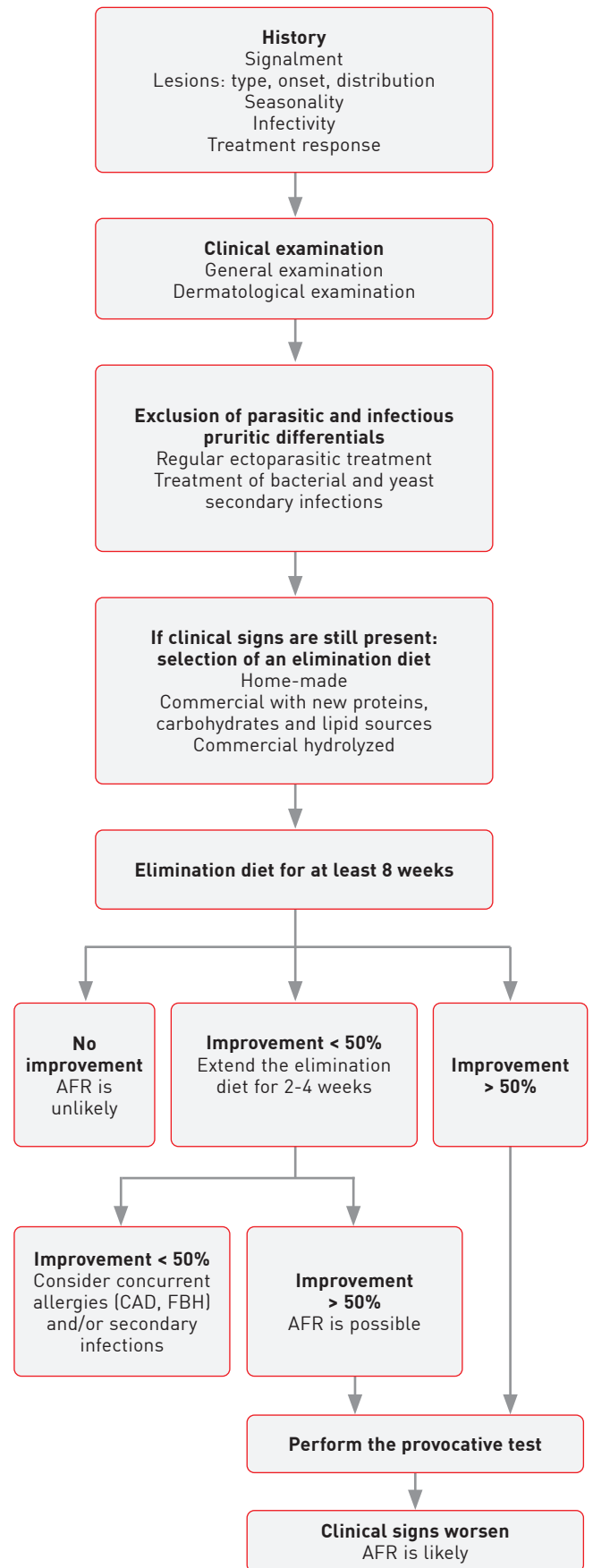**Clinical signs worsen**
AFR is likely

Figure 9. A schematic diagram showing the differential diagnosis of AFR.

glucocorticoids should be avoided in this scenario wherever possible. Oclacitinib should be given at the same dosage as for acute flares twice daily for 14 days and then once daily thereafter. Cyclosporine should be administered at 5 mg/kg q24H until clinical signs are alleviated, then tapered to a dose that maintains remission. A recently introduced alternative is lokivetmab, a caninized monoclonal antibody (mAb) which targets IL-31 (20). Given as a single injection once a month it has been shown to produce rapid alleviation of clinical signs, with decreased pruritus within a day of administration and a lessening of lesions within 7 days (21).

Oral essential fatty acids (EFAs) are of little use when treating acute flares due to the length of time needed for any possible beneficial effect to occur, although they do offer a glucocorticoid-sparing effect if used long term. Other drugs (*e.g.*, masitinib, recombinant canine interferon-gamma) appear to provide little or no benefit, and in any case their use is generally off-label when employed for this situation (22). Drugs such as high-dose oral pentoxifylline, oral low-dose weekly methotrexate, and adjunctive drugs including vitamin E and antihistamines have not been studied in detail and require further proof of efficacy.

It is also important to check for bacterial and yeast infections on the skin and ears whenever acute flares are triggered. If diagnosed, topical antimicrobial shampoos and sprays or, if necessary, appropriate topical and/or systemic antibiotics should be administered following national antimicrobial treatment guidelines (18,23-24).

Finally, sublingual immunotherapy has recently been investigated as a possible treatment for canine AFR, and at least one study has shown that it can safely induce clinical desensitization (25), so in future this option may help induce tolerance, preventing dogs from accidental exposure to food-specific allergens.

CONCLUSION

Dogs are prevalent to adverse food reactions (AFR) and although they can demonstrate typical clinical signs in typical locations, these are unfortunately not pathognomonic, and other diseases can manifest in the same way. To complicate matters, affected dogs may also have non-food-induced atopic dermatitis and flea bite hypersensitivity, and AFR can cause other problems, either alone or along with skin lesions. Diagnosis is based on clinical history, appearance, exclusion of other differentials and an elimination diet trial. Strict food allergen avoidance is curative (although accidental exposure can cause recurrence of clinical signs, requiring symptomatic treatment), but when the culprit allergens cannot be identified, long-term medication and dietary management are necessary to prevent relapse.

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ISOXAZOLINES FOR CANINE DEMODICOSIS

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There has been an influx of new molecules for treating canine ectoparasites over the last few years; here Vincent Defalque discusses the use of one of the most promising categories – the isoxazolines – for the treatment of canine demodicosis.

KEY POINTS

1 Isoxazolines are a new class of ectoparasiticides recently introduced to the veterinary market; they are effective and safe, with very few adverse reactions reported.

2 Isoxazolines have shown impressive results in controlling canine demodicosis over the last few years and are likely to be the mainstay therapy for many years to come.

Introduction

A new class of ectoparasiticides, isoxazolines, were first released in Canada in 2014, with afoxolaner and fluralaner tablets initially licensed only for the treatment of fleas and ticks in dogs. Anecdotal reports soon suggested that the new drugs were also effective against other ectoparasites, but scientific evidence for any proven efficacy of isoxazolines when used off-label in dogs suffering from other parasitic conditions – such as demodicosis – was slow to emerge, although this is now changing. This paper offers a brief overview of the new class of drug and its efficacy against canine *Demodex* mites.

Canine demodicosis

Demodicosis is caused by the proliferation of *Demodex* spp. mites and is a common disease in canine practice worldwide, with a variety of diagnostic and therapeutic options. Background information can be found in the WAVD Clinical Consensus Guidelines[1] which provide current information on the pathophysiology, diagnosis and treatment of commonly encountered dermatological conditions (1). Until recently, the drug of choice for many clinicians when faced with demodicosis was ivermectin, which was far from ideal as a first-line therapy.

However, this has changed since the introduction of isoxazolines. We all have memorable patients, and this was certainly the case with Kenny, a 6-month-old intact male Australian Shepherd who presented in September 2015 with severe dermatological signs and was diagnosed with demodicosis. His cutaneous lesions were predominantly facial **(Figure 1)**, although there was also a significant secondary bacterial pyoderma caused by methicillin-resistant *Staphylococcus pseudintermedius*. The severe pruritus induced by the pyoderma required Kenny to wear an Elizabethan collar 24/7, as he had self-traumatized to the point that there was total hair loss on his muzzle and periocular region. I was skeptical that a single dose of an oral medication administered to a dog with demodicosis could lead to a parasitological and clinical cure when it usually took several weeks of daily oral ivermectin to achieve remission. Nevertheless, considering the patient's age and – most importantly the breed, which is well known to be sensitive to ivermectin – this was an excellent opportunity to treat my first case of canine demodicosis with a single

[1] https://wavd.org/continuing-education/consensus-guidelines

Figure 1. Kenny on day 0. A single dose of oral fluralaner was administered at this point.
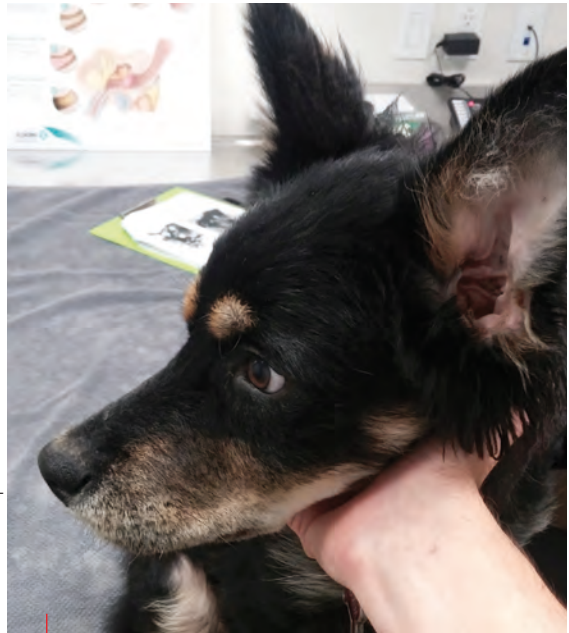
Figure 2. Day 44; Kenny showing full hair regrowth.

dose of oral fluralaner. When I saw Kenny for subsequent rechecks, the results were astonishing. By day 44 there was complete resolution of the skin lesions **(Figure 2)**, and there was no doubt that the drug was both effective and well tolerated. This then raised two important questions. Was it finally possible to relegate the use of titrated ivermectin and its occasional but alarming adverse effects to the history books? Were isoxazolines truly going to be a game changer for the treatment of canine demodicosis, especially for animal shelters and in communities with limited access to veterinary care? The answer to both questions was "yes" – and since then these drugs have shown impressive results in controlling canine demodicosis, such that they are likely to be the mainstay therapy for many years to come. Our bottle of ivermectin we had at the time ended up expiring at the back of a shelf.

Isoxazolines: a new class of drug

This new class of ectoparasiticide now encompasses various compounds, including afoxolaner, fluralaner, lotilaner, and sarolaner (2). These molecules have a novel mode of action, specifically blocking insect and acarine ligand-gated chloride channels. They act on the gamma-aminobutyric acid receptor (GABA) and glutamate receptors, inhibiting GABA and glutamate-regulated uptake of chloride ions, resulting in excess neuronal stimulation and rapid parasite death (3).

Isoxazolines are now commercially available either as oral or topical spot-on medications, with the original products containing a single active ingredient. In most countries these products are labelled only for the treatment, prevention and control of fleas and ticks, although some countries now have license indications that include other canine diseases, such as demodicosis, scabies, and otoacariasis. More recently, combination products containing an isoxazoline and a macrocyclic lactone (sometimes also with pyrantel embonate) have also become available and are labelled for the treatment and prevention/control of fleas and ticks, as well as other important endo- and ectoparasites.

Given that a variety of isoxazolines are now available in most countries, a short literature review of the most common compounds used to treat canine demodicosis may be helpful. 18 recently published studies have been identified that review the four commercially available isoxazolines, and the results in eradicating *Demodex* spp. mites are very encouraging. The efficacy of oral afoxolaner has been evaluated in 253 dogs in seven studies (4-10),

"Isoxazolines truly seem to be a game changer for the treatment of canine demodicosis, allowing us to relegate the use of ivermectin and its occasional but alarming adverse effects to the history books."

Vincent E. Defalque

whilst oral and topical fluralaner has been assessed in 371 dogs in eight studies (11-18), with the details summarized in **Tables 1 and 2** respectively. There are also reports on two of the other products in this drug category. Oral lotilaner has been evaluated in one study, where ten dogs treated three times 28 days apart (20 mg/kg PO) were all being mite-free at day 70, with no adverse effects noted (19). Two controlled studies have also investigated the efficacy of oral sarolaner. In one study 8 dogs were treated three times 30 days apart (2 mg/kg PO) with sarolaner alone, whilst another 8 dogs were treated with a weekly spot-on containing imidacloprid and moxidectin (20). All dogs were mite-free at day 44 and there were no adverse effects, but the oral sarolaner was reported to have performed better than the spot-on. The second non-inferiority study compared the same two products (21); 53 dogs were treated 30 days apart (2-4 mg/kg PO) with all dogs being mite-free at day 150, whilst another 28 dogs were treated weekly or monthly with the imidacloprid-moxidectin spot-on. There were no adverse effects with oral sarolaner, and again this drug performed better than the spot-on product.

Guidelines for treating demodicosis

The clinician should always follow the recommended labelled dosage for flea prevention/control / treatment when prescribing an isoxazoline for demodicosis, and conform to the minimum age and bodyweight requirements on the label. For certain oral products the drug bioavailability may be compromised if a dog is fasted before dosing, so fluralaner and lotilaner should be administered with food; the plasma levels of afoxolaner and sarolaner are the same regardless of whether the drug is given with or without food. My current preferred therapeutic option for a dog with demodicosis is to administer a single oral or topical dose of fluralaner, for two reasons. Firstly, there is a higher level of evidence for efficacy, as to date there are more controlled studies with this compound than the other products, and secondly the sustained 12-week duration of action has been linked to better owner compliance compared with the use of monthly treatments (22). However, there

Table 1. Afoxolaner studies.

Type of study and reference	Treatment protocol and outcome
Controlled study – 8 dogs (4)	• 3 doses 14 days apart and a fourth dose 28 days later (≥ 2.5 mg/kg PO) • 100% mite free at day 84 • No adverse effects • Another 8 dogs were treated with a spot-on containing imidacloprid and moxidectin (same intervals) • The isoxazoline performed better than the spot-on therapy
Case series – 4 dogs (5)	• 3 doses 28 days apart (≥ 2.5 mg/kg PO) • 100% mite free at day 56 • Adverse effects not recorded
Case series (unpublished) – 102 dogs (6)	• Treated every 2 to 4 weeks (≥ 2.5 mg/kg PO) • 100% mite free at day 90 • Adverse effects not recorded
Case series – 6 dogs (7)	• 1, 2 or 3 doses; 21, 28, 35 or 42 days apart (2.7-5.6 mg/kg PO) • 100% mite free at day 77 • No adverse effects
Case series – 15 dogs (8)	• Treated with a combination of afoxolaner-milbemycin oxime • 3 doses, 28 days apart (2.5-6.3 mg/kg PO) • 99.9% mite reduction at day 84 • No adverse effects
Case series – 50 dogs (9)	• Treated with afoxolaner (31 dogs) or the combination of afoxolaner-milbemycin oxime (19 dogs) • 3 doses, 28 days apart (2.5-2.7 mg/kg PO) • 98% mite reduction at day 84 • Adverse effect: vomiting (1 dog)
Case series – 68 dogs (10)	• Treated with a combination of afoxolaner-milbemycin oxime • Single dose (2.50-5.36 mg/kg PO) • 82.4% mite reduction at day 28 • Adverse effects not recorded

Table 2. Fluralaner studies.

Type of study and reference	Treatment protocol and outcome
Controlled study – 8 dogs (11)	• Single dose (≥ 25 mg/kg PO) • 100% mite free at day 56 • No adverse effects • Another 8 dogs were treated with a spot-on containing imidacloprid and moxidectin (3 doses, 28 days apart) • Oral fluralaner performed better than the spot-on
Case series – 163 dogs (12)	• Single dose (≥ 25 mg/kg PO) • 100% mite free at day 60 • No adverse effects
Case series – 4 dogs (13)	• 2 doses, 60 days apart (≥ 25 mg/kg PO) • 98% mite reduction at day 90 • Adverse effects not recorded
Case report – 1 dog (14)	• Single oral dose • 100% mite (*Demodex injai*) free at day 49 • Adverse effects not recorded
Case series – 67 dogs (15)	• 1 to 3 doses, 84 days apart (25-50 mg/kg PO) • 100% mite free at day 90 • No adverse effects
Case series – 20 dogs (16)	• Single dose (25-56 mg/kg PO) • 100% mite free at day 56 • No adverse effects
Controlled study – 8 dogs (17)	• Single topical spot-on dose (≥ 25 mg/kg) • 100% mite free at day 84 • No adverse effects • Another 8 dogs were treated with a spot-on containing imidacloprid and moxidectin (at weekly to monthly intervals over 84 days) • The spot-on fluralaner performed better than the imidacloprid/moxidectin spot-on
Controlled study – 100 dogs (18)	• Single oral or topical spot-on dose (25-56 mg/kg) • 100% mite free at day 84 (oral) and 98% mite free at day 84 (topical spot-on) • No adverse effects • Another 24 dogs were treated with a spot-on containing imidacloprid and moxidectin (at weekly to monthly intervals over 84 days) • Oral and topical spot-on fluralaner performed better than the imidacloprid/moxidectin spot-on

are alternatives. One is to give a single oral dose of afoxolaner (or the afoxolaner/milbemycin oxime combination) once a month for three months. This regime is also suitable if using oral sarolaner (or the sarolaner/moxidectin/pyrantel embonate combination) or lotilaner. In most cases systemic antibiotics will not be needed; topical antibacterial therapy combined with good miticidal agents will be sufficient unless a severe bacterial infection is present (1).

However, it is prudent to sound some words of caution when treating *Demodex* infection with these compounds. Geographical differences exist in the availability and licensure of isoxazoline drugs for use in dogs, and the clinician must consider the relevant regional prescribing recommendations. If prescribing an isoxazoline to treat canine demodicosis represents an extra-label use, some national or regional legislation will permit such therapies only where a drug licensed to treat canine demodicosis has either failed or is contraindicated (1). In addition, adult-onset generalized demodicosis may be associated with an immunosuppressive condition or concurrent treatments for other medical conditions, so recurrences are possible regardless of the product used. Animals being treated for generalized demodicosis should be monitored clinically and microscopically every month until the second negative skin scraping, and a follow-up of at least 12 months after treatment cessation has been

recommended before we can say a dog is cured (1). Finally, although isoxazolines are generally very safe, they can occasionally have potential side effects, including vomiting, diarrhea, anorexia, lethargy, and seizures; in particular, if using this class of drug in a dog with a history of seizures or other neurological disorders the clinician should always proceed with caution. Essentially, isoxazolines should only be used in suitable patients and always under veterinary supervision.

CONCLUSION

The recent introduction of isoxazoline ectoparasiticides has resulted in an apparently effective and safe therapy for canine demodicosis – a condition that has been traditionally difficult and frustrating to treat – with low frequency of administration. It seems that ivermectin can now be discarded and the clinician can be more confident in selecting a product that will benefit canine patients, although given that this class of drug may not have a national license for use in demodicosis, appropriate precautions should still be taken when prescribing it.

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WOUND MANAGEMENT WITH COLD PLASMA THERAPY

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KEY POINTS

Cold plasma therapy is a simple, painless treatment method that efficiently eliminates infectious agents and accelerates the wound-healing process.

1

Although CAPP can be very effective against multi-resistant bacteria, it does not eliminate any underlying cause, and must not replace a clinical diagnosis.

2

Cold atmospheric pressure plasma therapy is an emerging technology in the veterinary field; this paper offers an introduction to the novel procedure and how it can benefit the canine patient.

Introduction

Given the worldwide rising number of drug-resistant bacterial and fungal infections, it is becoming increasingly important to develop alternative treatment options for such infectious pathogens. Progress towards sustainable physical or other methods that can eliminate such problematic agents appears ever more crucial, and Cold Atmospheric Pressure Plasma (CAPP) Therapy is such a procedure, offering proven efficiency in treating

Figure 1. CAPP therapy using an argon gas cold plasma pen for ulceration of a dog's pinna.

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antibiotic-resistant bacterial, viral and fungal pathogens (1-5). The technique also modifies and upregulates numerous factors that promote and accelerate healing, which can especially benefit patients with wound-healing disorders (6,7). Originally used in human medicine, CAPP is now becoming more widely accepted in veterinary medicine, partly because it is a painless procedure that can be applied without sedation (8), although the current lack of animal studies means that the technique is still relatively unknown. This article provides an insight into the therapy and some practical examples of how it may be used effectively in small animal clinics **(Figure 1)**.

Basic physical principles and mode of action

Plasma is sometimes called the "fourth state of matter" (after solid, liquid and gas), and is essentially a gaseous mixture of free ions or electrons within a confined space (9). Natural examples of the phenomenon include lightning and solar flares, but plasma can also be produced artificially at room temperature and under normal atmospheric pressure, for example by accelerating charged gaseous particles along an electromagnetic field. CAPP

Figure 2. A portable cold plasma pen which uses the skin as the anode for plasma generation. Small flashes of light between the device and the lesion are visible.
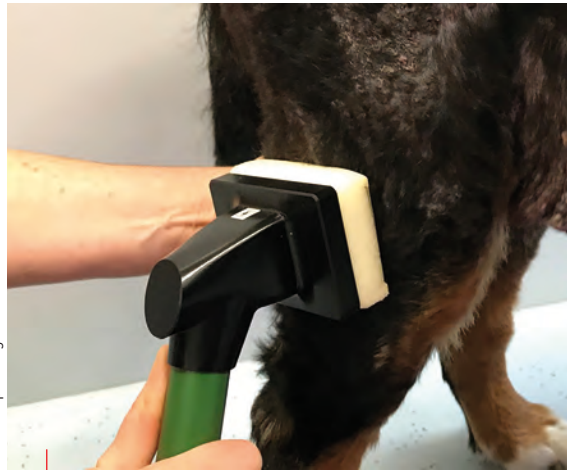
Figure 3. Some devices use foam to provide a wide surface area, ideal for treating large lesions.

therapy has been shown to positively influence tissue healing by hastening the healing process and reducing scar formation. How it produces its effects is as yet not fully understood, although it is known that CAPP strongly influences certain growth factors (*e.g.*, FGF-7 for keratinocyte migration), anti-inflammatory signaling molecules (*e.g.*, TGF-β) and inflammatory signaling pathways (6-11).

CAPP was initially reserved for wound disinfection and to promote healing in human burn victims, but is now indicated for use in many other situations. It is effective in treating both simple and complicated skin infections (especially where multi-resistant pathogens are present) as well as for various other wound-healing disorders, such as those that can develop secondary to diabetes (1,3,6). The therapy is widely reported to be highly effective in combating bacterial, viral and fungal pathogens, even where there is biofilm formation (2,3,5,9), and its physical mode of action means that any

resistance to antibiotics, antimycotics or antivirals is irrelevant. Studies have shown that CAPP has an excellent bacteriostatic effect on methicillin-resistant *Staphylococcus aureus* spp. (*MRSA*), *S. pseudintermedius* (*MRSP*) and multi-resistant *Pseudomonas aeruginosa* (*MRPA*), some of the most common bacterial skin pathogens in veterinary medicine (1-4).

## Device design and application

Currently, there are three basic types of device available, each with certain advantages and disadvantages. All involve the creation of cold plasma by ionizing a gas into its plasma state, usually either atmospheric air (*i.e.*, oxygen and nitrogen) or an inert gas such as argon.

1. The simplest and cheapest type (from €2,000) creates an electric charge on the device cathode and uses the skin as the anode, with plasma being generated in the narrow space between the two **(Figure 2)**. The main advantages – other than cost – are the simplicity of use and a comparatively simple design, which allows the device to be battery-powered. Some patients find the noise or the "tingling" sensation, which depends on the intensity of the current, unpleasant.

2. A second type of device uses an intermediate medium (*e.g.*, foam) placed between the cathode and the skin as an electrical conductor. This lessens or eliminates any tingling sensation **(Figure 3)**, although the direct wound contact can still be perceived as unpleasant by some patients. This method can treat a relatively large surface area and allows efficient use of time if treating larger wounds or big dogs. However, for small patients, smaller wounds, or for skin fold lesions, correct placement of the foam can make application more difficult. In addition, new pads are required for each patient, and although the devices are portable, they require mains power to function.

"CAPP therapy is widely reported to be highly efficient in combating bacterial, viral and fungal pathogens, even where there is biofilm formation, and its physical mode of action means that any resistance to antibiotics, antimycotics or antivirals is irrelevant."
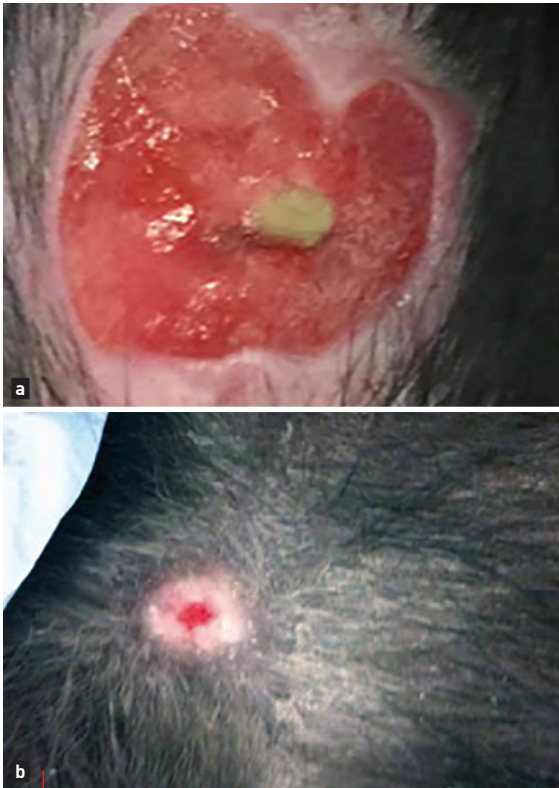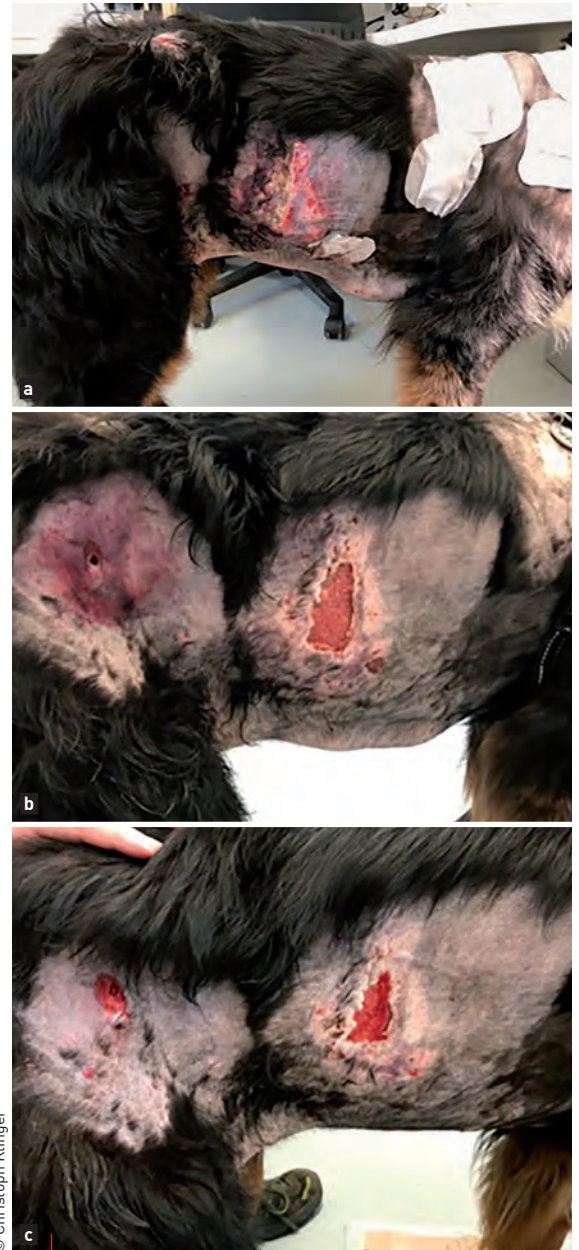
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Figure 4. The concave aspect of the pinna in a four-year-old Labrador with leishmaniasis with ulceration down to the cartilage on day 0 **(a)** and at day 28 after CAPP **(b)**.

3. A third type of device generates plasma from an inert gas such as argon which is then released at the tip of the treatment pen as a small flame or "jet" **(Figure 1)**. The jet is passed over the skin surface in circular movements, close but not touching the wound itself. This design allows selective "spot" treatments, even in deeper skin folds or wound cavities, and can enable rapid drying of weeping and purulent wounds with very little irritation or noise. The disadvantage lies in the high purchase cost (up to €15,000), the gas consumption, and the significantly limited portability of the device.

All three options are easy to use and can be operated by assistants after a brief instruction period, allowing CAPP therapy to be conveniently integrated into the daily practice routine, either under non-sterile conditions in a consulting room, or in an aseptic operating theatre. Since it is painless the patient rarely requires sedation or anesthesia, although success obviously depends on identifying the root cause of the problem (6,7). The duration and frequency of application depends partly on the device specification (with the penetration depth varying from nanometers to a few millimeters) and the type, depth and nature of the lesion. Typically, treating an affected area every 2 or 3 days for two weeks, and then decreasing to once weekly, has proven to be a generally effective initial regimen.

To date, side effects of CAPP appear minimal, other than minor skin irritation where there has been prolonged skin contact (8). Whilst there have been

Figure 5. Necrotizing fasciitis in an eight-year-old immunosuppressed Bernese Mountain dog treated with CAPP, from day 0 **(a)**, day 7 **(b)** and day 11 **(c)**.

few comparative studies to review the efficacy of the different devices (12), the author believes that patient tolerance and the speed of healing appears to be best with the third design. However, owners have generally been very satisfied with the results from any of the CAPP devices and have been willing to pay the additional costs involved for this therapy.

●●● Possible veterinary applications

At present all devices are designed primarily for topical use, and the most significant and innovative aspect of CAPP therapy is that it achieves physical

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Figure 6. Perianal fistulae in a three-year-old German Shepherd dog before treatment **(a)** and at day 18 **(b)**. Note that only the left half of the anus was treated with CAPP.
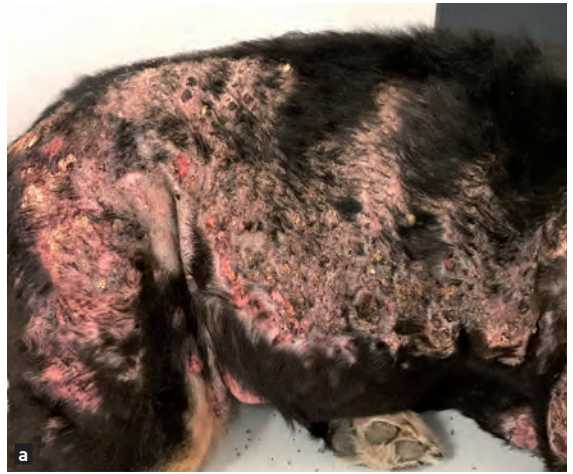
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Figure 7. Severe iatrogenic calcinosis cutis in a four-year-old Bernese Mountain dog with pemphigus foliaceus **(a)**. The same dog at day 28 following CAPP treatment **(b)**.

disinfection of almost any site that has bacterial, viral or fungal involvement (1,4,5), and is highly effective against both non-resistant and resistant bacterial strains (1,12). Given its limited tissue penetration, open, shallow wounds appear to be the ideal application for the technique; its beneficial effects in hard-to-reach areas (*e.g.*, interdigital clefts, body cavities, auditory canals and deep wounds) are more questionable. At least for now, much depends on the device design and the type of lesion treated, so some CAPP devices may be well-suited for treating pododermatitis or otitis externa, whilst others are more suited for use on large surface areas.

Other than the disinfection aspect, other benefits are also becoming apparent for this therapy. For example, it is increasingly being used in vasculitis-related lesions such as those seen in leishmaniasis. **Figure 4** shows an affected Labrador that had previously received a four-week course of meglumine antimoniate, miltefosine and allopurinol. While both the clinical parameters and antibody titers responded well to treatment, the associated vasculitis led to gradual worsening of ulceration of the inner aspect of the

pinnae, with exposure of the underlying cartilage. This was brought into almost complete remission within 28 days using CAPP therapy, although signs reappeared six months later due to the associated leishmaniasis.

Importantly, although CAPP will promote wound healing, recurrence is likely to occur within a short time frame if the underlying disease is not treated as well, for example with immunosuppressed patients (13). **Figure 5** shows an eight-year-old Bernese Mountain dog that developed septicemia secondary to a necrotizing foreign body ileus. The dog had previously been diagnosed with hypoadrenocorticism and had been treated with deoxycorticosterone for several years. As a result of the septicemia the patient developed necrotizing fasciitis at multiple sites on the flanks, which had shown limited response to triple antibiotic treatment, presumably due to the corticosteroid therapy. However, CAPP application produced a rapid improvement over a three-week period, and although the dog developed additional areas of fasciitis during this time, these were also successfully treated, and all lesions were resolving after 24 days of treatment, with no further recurrence.

CAPP has also been shown to be beneficial in patients with various immune-mediated diseases. This is demonstrated in **Figure 6** which shows a three-year-old German Shepherd dog with perianal fistulae. The dog was treated with a combination of CAPP, cyclosporine and topical tacrolimus, but for comparison only the left half of the anus was treated with CAPP, with the right side covered by paper during the cold plasma sessions. After 18 days it was evident that whilst medication was effective, the left side showed significantly faster wound closure and less scarring than the other side.

Another current focus is the beneficial effect of cold plasma therapy on fibrosis (11). **Figure 7** shows a four-year-old Bernese Mountain dog with severe calcinosis cutis secondary to iatrogenic hyperadrenocorticism, the result of treatment for pemphigus foliaceus. Apart from local anti-inflammatory therapy (*e.g.*, DMSO) and switching from glucocorticoids to alternative drugs such as cyclosporine to control the pemphigus, treatment options in such cases are very limited. Cutaneous calcinosis can often lead to significant scarring, but here CAPP treatment resulted in a very rapid response, with 90% of the skin fully healed and without scarring within four weeks, and with subsequent complete hair regrowth.

Finally, for now, CAPP may have an application in other areas. Research is already being conducted into options that will allow the technique to be applied internally via minimally invasive interventions (*e.g.*, by endoscopy) (14). Its use in surgical cases is still controversial; it may be beneficial for postoperative wound disinfection and scar prevention, but uncertainty exists regarding its use intraoperatively; although it may reduce the bacterial load from surgery, the prolonged operating time it necessitates may allow fluid loss from tissues, leading to poorer healing (11,15).

CONCLUSION

Cold atmospheric pressure plasma (CAPP) therapy is a simple physical treatment that can significantly hasten healing for many skin wounds. It efficiently eliminates infectious agents regardless of any drug resistance, and accelerates patient recovery, especially where there are factors that could slow the healing process. Because application is quick, painless and uncomplicated, it is also well-suited for everyday use in the practice, although a definitive objective assessment of its efficacy is still lacking. Importantly, CAPP therapy should not replace careful diagnosis by the veterinarian, as it cannot cure any underlying disease.

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