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.

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

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

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.

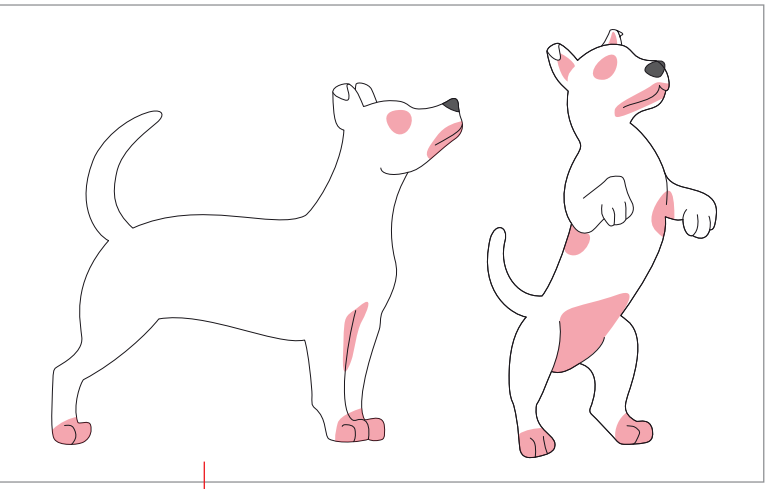

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Figure 2. The red coloration depicts the most typical predilection sites for canine atopic dermatitis.

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

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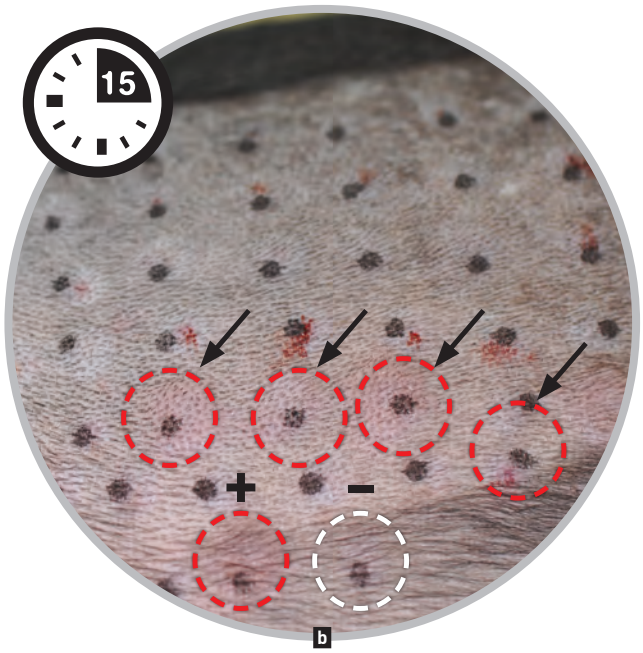
INTRADERMAL SKIN TESTING

Injection of allergens



Interpretation of results

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.

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.

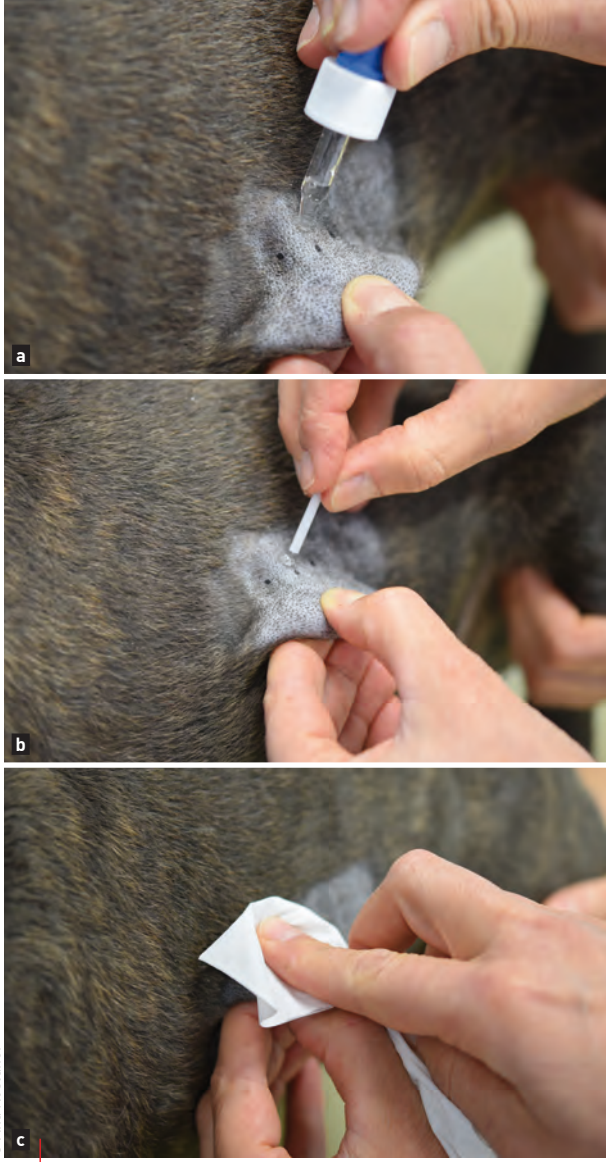


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Figure 4. Performing a skin prick test in an atopic dog for the house dust mite allergen *Dermatophagoides farina*. This can be done without sedation, with the dog in a standing position and the flank shaved as for an IDT. **(a)** One drop of the environmental allergen is applied to the skin. **(b)** The skin is immediately pricked using a commercial device held at 45^0 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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