What is an "allergy test"?

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Summary abstract

Allergen specific IgE serology may be used in atopic dogs with well-established all year round clinical disease as a means of determining potentially significant allergens for inclusion in immunotherapy. Atopic dermatitis is diagnosed on the basis of appropriate clinical signs and history and on ruling out other causes of pruritus.

Serological tests for food and Malassezia allergens are not recommended. Tests may be influenced by steroid therapy and should not be used as part of an initial diagnostic "work up". Given that normal healthy dogs can have IgE antibodies to environmental allergens and atopic dogs may not, then it is critical to understand and accept that these are not diagnostic tests. Furthermore, the lack of standardisation and external validation of the allergens, reagents and reporting methods means that all test results should be interpreted with caution.

Immunotherapy is helpful in some atopic dogs and can be pursued with motivated owners who understand the potential value of this safe treatment modality. Given that immunotherapy can take many months to change the pattern of skin disease observed through the year it is important to accept that any testing is primarily a direct route to pursuing immunotherapy. Consequently, such testing (intradermal and serology) may not be suitable for every case of canine atopy and should only be explored once the pattern of the skin disease has been observed throughout most of the year in order to appreciate any seasonal variations.

Introduction

Veterinary clinicians and owners have access to a variety of commercial serological tests that purport to measure IgE and, in some situations, IgG antibodies, to various allergens. It is clear from doing referral case work and providing advice to clinicians that there is considerable confusion about how to use these tests and that there is great potential for misusing these tests and for wasting clients' money. The aim of this article is to provide an overview of how these tests can be used in dogs; the use of such tests in cats and horses is more complex and the reader is directed to other sources (Noli and others, 2014).

When discussing serological methods and atopic dermatitis it has long been emphasised that the term "allergy test" is potentially misleading; they are not definitive diagnostic tests, they are meant to aid diagnosis and therapy (DeBoer and Hillier, 2001). These tests are to be used when considering a case of canine atopic dermatitis; this disease has well defined historical and clinical criteria (Hensel and others, 2015); serological (and intradermal) tests are not required to make a diagnosis. The term allergen test will be used hereafter (short for allergen-specific IgE serology). Similar constraints also apply to the use of intradermal tests).

Atopic dermatitis in dogs is usually diagnosed on the basis of an appropriate history and clinical signs, ruling out ectoparasites and considering the role of cutaneous microbial infections and assessing for flea, food and contact allergy. Many healthy normal dogs can have allergen-specific IgE antibodies; the term subclinical sensitization is sometimes coined when this is reported but this is potentially misleading because it could be inferred that the dog could be sub clinically allergic – when in reality normal dogs can have a range of antibodies to antigens in their environment. Furthermore, substantial numbers of atopic dogs do not have allergen-specific IgE in their skin or serum – but they still fulfil the criteria for being atopic (the terms "atopic-like dermatitis" and "intrinsice atopic dermatitis" are sometimes used to describe these dogs).

Consequently, allergen tests should only be used in dogs well after their skin disease is already definitively diagnosed as consistent with atopic dermatitis. It is not clinically appropriate to use such tests when starting to investigate a dog with skin disease (sometimes euphemistically called

a "full derm work up"); the use of such tests is going to be cost effective only after the case has been thoroughly investigated and the disease pattern established.

Components of an allergen test

Components of an allergen-specific serological test and their limitations	
Allergen	Allergens are commercially prepared for veterinary use; the selection of the allergens is based in part on allergens used in studies of human allergic conditions and in part on previous positive test results. Allergens are extracts and are potentially composed of a large number and wide variety of antigenic components. In human allergy the allergens have been highly characterised. Studies in dogs have attempted to characterise some of the environmental allergens to which they may be exposed, however, there is limited knowledge at present (Mueller and others, 2016). In laboratory testing the allergen extracts are not standardized, making calibration and evaluation of the test results problematic.
Serum	Diluted dog serum is added to the allergen and incubated
Anti-IgE reagent	Historically it was assumed that this component of the test was unreliable because of cross reactivity with the much more common IgG antibodies in dog serum. In recent years regents have been shown to be more specific and may include monoclonal antibodies and recombinant proteins (Fc-epsilon components).
Signal molecule	This is a reagent that binds to the previous reagent, that has bound to the IgE and provides an output that can be measured usually in optical units.
Reporting mechanism	There are no internationally recognised standard reference units for the measurement of IgE; consequently, all tests report arbitrary (relative antibody) units that are usually calibrated against results derived from (undefined) healthy and allergic cases, and modified by intra-assay standards. There are no independent recognised reference standards for these assays. There is no external regulation of these tests in terms of standardization or calibration or quality control. There are published reports of the reproducibility of these tests but that does not tell us anything about their reliability or clinical merit in clinical practice. It is not possible to readily compare results from different laboratories because they are potentially using different antigen extracts, assay conditions, reagents and reporting systems.

Allergens

Allergen type	Comments
Flea	The pathogenesis of flea allergy is presumed to involve a variety of immune mechanisms including type 1 hypersensitivity reactions associated with IgE; type 4 delayed hypersensitivity reactions may also be involved. It is important to appreciate that the detection of antibodies to flea allergens does not prove that the dog is allergic – healthy normal dogs can also have such antibodies. Positive test results can be used to support a flea control programme and convince the owner that flea exposure has taken place. Negative test results do not rule out exposure or the possibility of flea allergic dermatitis.

Food	Considerable efforts have been expended to find a serological test that can readily enable a diagnosis of cutaneous adverse food reaction. The pathogenesis of "food allergy" remains far from clear and it is unlikely to involve IgE alone. Systematic review of the literature **does not** support the use of serological tests in the diagnosis of food allergy. There is no good quality evidence that such tests can help to select a diet for the exploration of dietary allergy. Current recommendations are to pursue a novel protein-based or hydrolysed diet for at least eight weeks (Olivry and others, 2015; Mueller and Olivry 2017).
Malassezia	Atopic dogs will respond to therapy for Malassezia infection with improvement in the lesions and the pruritus. This could suggest that in some way Malassezia organisms are involved in an allergic process in the dog. While intradermal and serological methods have been used to detect antibodies to Malassezia extracts their clinical significance is unclear. Evidence that dogs may respond to immunotherapy with Malassezia extracts is limited at present. When assessing Malassezia infection it is important to use cytology and culture methods to demonstrate the presence of infection and then treat accordingly.
Mites	House dust and storage mites are the most important allergen group for atopic dogs and such allergens are ubiquitous and difficult to avoid in the home environment. Such cases are usually associated with pruritus that is present all year round. Some dogs may be worse in the summer months because of warmer conditions and higher exposure to dust mite allergens.
Pollens	It is often assumed that dogs are allergic to pollens but the pattern of skin disease in atopic dogs in the UK is usually not seasonal – making it unlikely that pollens are playing a role. Some atopic dogs only show signs during the summer months and these cases can be positive on testing for pollens, supporting a role for pollen allergy.
Moulds	The evidence that dogs are sensitive to mould allergens is limited. When testing a dog with all year round signs of disease it may be the case that moulds are clinically important if they come up positive in a test.
Sarcoptes	The test for exposure to scabies mites is not strictly an allergen specific IgE test because it is usually an IgG ELISA methodology (reviewed by Arlian and Morgan, 2017). Some authors believe that the test is useful – although like allergen testing there is no external validation of the methodology. Published studies for dogs suggest high specificity and that dogs seroconvert in 2-4 weeks after initial exposure. So, a positive test is supportive of a diagnosis. However, it could be contended that with various licensed products based on macrocyclic lactones and isoxazolines, that ruling out scabies in a very pruritic dog is fairly straight forward. That is, when you suspect scabies it will not really matter what is the test result – the dog ought to be treated to rule out scabies (especially when they are very pruritic).

Allergen avoidance

It is impossible for atopic dogs to completely avoid the allergens that are causing their skin disease. Moving a dog indoors may reduce exposure to pollens but there is no evidence that this is sufficient to control the skin disease alone. Moving a dog outdoors may enable a significant

reduction in exposure to house dust and storage mites, but most owners cannot keep their pet completely out of the home. Atopic dogs that seem to improve when placed in kennels, while the owners are on holiday for several weeks, may have benefitted from reduced mite exposure away from home.

Given the propensity for dogs to be sensitised to mites there have been various suggestions for reducingallergen exposure and some are given below; unfortunately it is highly unlikely that these measures will have a dramatic impact on the pruritus.

a) Prevent dust accumulation by removing 'clutter' from sleeping areas for example toys, chews and excess bedding.
b) Keep dogs out of the human bedrooms and away from carpeted areas if possible.
c) Wash bedding regularly (weekly) on a hot cycle.
d) Decrease the temperature of sleeping areas and increase ventilation.
e) Frequent vacuum cleaning and dusting.

Some laboratories recommend using household flea sprays for mite control – this will not be highly effective alone in controlling mite allergen exposure because the allergen source is still present in the home (albeit as dead mites(; it may be supportive of other management control methods (and help to reduce the risk of flea exposure).

Laboratories also recommend feeding wet food for dogs testing positive to storage mites because these mites have been recorded as present in dry dog food (Hibberson and Vogelnest, 2014)). There is no substantial benefit from this approach and the positive serology test results merely reflect exposure and some degree of cross reactivity to house dust mites. Furthermore, the dogs that test positive will be exposed to much higher mite allergen levels in their home environment independent of the dry dog food bags.

The key point here is that allergen avoidance is worth pursuing but on its own it is unlikely to make the atopic dog substantially better.

Interpretation

Some test results can show multiple positive results which can be confusing and daunting to the owner and clinician.

For atopic dogs with non-seasonal, all-year-round disease, a positive result to house dust and storage mites may well be significant. One factor to take into account is that there may be cross reactivity to mite allergens and intriguingly discordant results. Many dogs will test highly positive for the house dust mite *Dermatophagoides pteronyssinus* (which is assumed to be the predominant dust mite in the UK); the same dog will often test even higher for *D. farinae* (which is not thought to be common in the UK). Even so, such results may still be used for immunotherapy. Many atopic dogs with positive test results to house dust mites will also test positive to storage mites; this is likely to be due in part to cross reacting antibodies attaching to common epitopes in the mite allergen extracts.

Non-seasonal cases with high test results for pollens are difficult to interpret when there is no obvious fluctuation in the skin disease during the various phases of the pollen season as tree, grass and weed pollens are shed from spring to summer (March to September). Some clinicians assume the results that are positive are significant. Given the large number of dogs that can test positive to pollens with non-seasonal disease it is potentially more important to focus on the environmental allergens that are driving the skin disease all year round – mites and possibly moulds. The pollen antibodies may merely reflect non-specific (or clinically insignificant) upregulation of the allergic dog's immune system. Furthermore, there are reports of cross reactive carbohydrate antibodies to protein-linked carbohydrate antigens in dog serum that may, as in humans, be clinically irrelevant because they lead to false elevation and may confound the results of serological testing in dogs (Levy & DeBoer 2018).

Dogs with only seasonal disease – usually in the pollen months – may only test positive to pollen – which could be significant. However, such testing is probably not prudent use of client/owner resources because such testing is mainly performed to pursue immunotherapy; it may be more cost effective and clinically efficacious to use various other therapies to manage the pruritus during the months of the dog's season of disease.

Influence of steroids

One of the proposed advantages of serological testing is that one does not have to stop steroid therapy before taking a sample and this is supported in part by a review of the influence of drug therapies on allergen testing – both intradermal and serological. It has to be stated, however, that there is limited data on the influence of steroids on allergen-specific IgE serology test results and the two studies mentioned by Olivry and others (2013) are either an older test method or only looked at one allergen; furthermore, some companies do believe there is an influence of steroids on their test system (Wassom and Grieve 1998). Given the substantial number of dogs that test negative with allergen tests it is prudent to be cautious about the influence of steroids on the test results and try to avoid long term steroid therapy immediately before a dog is sampled. The author uses the same wash out periods as those suggested for intradermal testing (empirically three weeks for oral steroids). One review reported withdrawal of oral steroids, such as (methyl)prednisolone, for two weeks prior to IDT, although the minimum withdrawal time could not be estimated with certainty (Olivry and others, 2013).

Intradermal tests

The intradermal test (IDT) has been used for many years to identify allergens for inclusion in allergen immunotherapy (AIT). While familiarity with this method may suggest that this is the "gold standard method for testing" it has to be acknowledged that the allergen extracts used in such tests are also not standardised or well characterised. Indeed, studies are still ongoing to try to establish irritant concentration thresholds to improve the veracity of such tests. The IDT can be used with allergen serology to potentially identify clinically significant allergens. They are detecting different types of IgE and are often not well correlated with one another; even so, the results for either test or when combined can be interpreted in the light of the clinical history.

Screening tests

Some laboratories offer screening panels where serum is tested against a group of allergens including various pollen groups and indoor mite allergens. There is no good quality evidence that these tests are useful in clinical practice – one would only need to use a serum test if intending to pursue immunotherapy and so the dog will have already been deemed to have met the criteria for being atopic and so these screens are not diagnostic. They are in reality a tool to promote the investigation of pruritic dogs and to use serology; they are not recommended.

Immunotherapy (AIT)

Allergen specific immunotherapy has been recommended for many years for atopic dogs and is available through several companies (DeBoer 2017; Mueller and others 2017); in the UK there are no licensed products so an importation certificate from the VMD is required. The success of immunotherapy is extremely variable and it is likely that many clinicians and owners abandon this approach at an early stage. Most cases are injected subcutaneously with incremental doses of allergen and reach a maintenance dose every few weeks. It can take up to 12 months to see the full impact of immunotherapy and in the interim it is important to intermittently stop other therapies to see if the immunotherapy is providing some control of the pruritus. There are protocols for AIT including sublingual and intralymphatic routes of administration.

Immunotherapy is usually employed in conjunction with a variety of other therapies (oral and topical) depending upon the nature of the dog's atopic skin disease. Owners have to understand and accept that atopic skin disease is a lifelong condition that requires constant regular intervention with AIT usually forming one part of the control measures for each case.

Immunotherapy products are usually based on the results of IDT and / or serology tests. They are attractive because they are usually extremely safe. Owners can be trained to administer the injections to their own pets. It is critical to choose clients and pets very carefully for immunotherapy – owners need to understand how immunotherapy should be used and when to seek veterinary help when the dog's skin condition is deteriorating – particularly when there are flare ups. In some cases immunotherapy can be a very cost effective and safe means of controlling the signs of atopic dermatitis.

Atopic skin disease can have variations in the pattern of the clinical signs observed during different seasons of the year. Consequently, immunotherapy ought to be given over many months, to see the full impact of such therapy on the pattern of the disease, to include any seasonal variations. It follows that any allergen testing (intradermal and / or serology) should only be performed long after the skin condition has started and not as part of an initial investigation when the skin condition initially becomes apparent. In the case of seasonal problems in the summer then the testing should be performed in the autumn in order to maximise the chances of identifying clinically significant allergens. Given the above it should be no surprise that most atopic dogs that are deemed suitable for testing will be well over one year of age and have had skin disease for six to 12 months before ANY allergen testing is considered.

While the pattern of the pruritic skin disease is developing in an atopic dog the owner and attending clinician can be kept very busy investigating other causes of pruritus including ectoparasites and pursuing diet trials; various therapies can be trialled to include the management of secondary microbial infections. These activities can all contribute to the successful management of an atopic dog. Performing IDT or taking a blood sample for allergen serology should then be seen as a route to immunotherapy and just one of the various interventions that may be used to manage atopic dogs. In that regard such testing should only be performed once the pattern of the skin disease is well established.

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MCQs

Which allergen group is the most important for atopic dogs?

1 Pollen
2 Moulds
3 Scabies
4 Dust mites

Allergen serology testing is useful for the management of which allergic skin condition in dogs?

1 Cutaneous adverse food reaction
2 Contact allergy
3 Atopic dermatitis
4 Flea allergy dermatitis

Allergen serology methods bind to what type of antibody in dog serum?

1 IgG
2 IgE
3 IgA
4 IgM

Allergen serology test results may be particularly useful in the management of canine atopic dermatitis using what type of intervention?

1 Allergen avoidance
2 Allergen immunotherapy
3 Identifying breeds at risk of developing skin disease
4 Identifying dogs at risk of developing skin disease