



# Prevalence of positive reactions in intradermal and IgE serological allergy tests in dogs from South Australia, and the subsequent outcome of allergen-specific immunotherapy

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**Objective** To determine the prevalence of positive allergen reactions in intradermal and IgE serological tests in dogs presenting to a dermatology referral centre in South Australia and the clinical efficacy of subsequent allergen-specific immunotherapy.

**Design** Retrospective study.

**Methods** Results from 108 intradermal allergy tests, 25 IgE serological assays and immunotherapy outcomes in 37 dogs were retrospectively analysed. Immunotherapy outcomes were determined as excellent, good, modest or failure using a global assessment of efficacy matrix which incorporated pruritus scores, lesion severity, medication requirements, and owner and clinician opinion.

**Results** The most common positive reactions in intradermal allergy tests were Red clover (59%), *Dermatophagoides farinae* (29%), *Tyrophagus putrescentiae* (28%), Yellow dock (25%) and *Malassezia pachydermatis* (24%). In the IgE serological tests, Yorkshire fog grass (40%), Yellow dock (36%), Kentucky bluegrass (36%) and *T. putrescentiae* (36%) were the most commonly reported positive results. The outcome of allergen-specific immunotherapy was judged to be excellent in 20% of dogs, good in 15%, modest in 18% and a failure in 47%.

**Conclusion** As has been reported in other geographical areas, environmental mites and plant pollens frequently gave positive reactions in allergy tests in South Australia. However, the prevalence of individual allergen reactions differed between intradermal and IgE serological tests, with *M. pachydermatis* being identified as a common cause of hypersensitivity in intradermal tests but not in IgE serological assays. Immunotherapy was judged to be a beneficial treatment in 35% of dogs but was essentially unsuccessful in 65%.

**Keywords** allergy; dog; IgE; immunotherapy; intradermal; South Australia

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Canine atopic dermatitis is a genetically predisposed inflammatory and pruritic allergic skin disease, with characteristic clinical features that is associated most commonly with IgE antibodies to environmental allergens.<sup>1</sup> A tentative diagnosis of atopic dermatitis can be made based on the presence of typical historical and clinical features, and after other similar pruritic dermatoses have been ruled out.<sup>2</sup> Allergy tests can be used to demonstrate

IgE-mediated hypersensitivity and are used to identify offending allergens for allergen-specific immunotherapy.<sup>2,3</sup>

Intradermal allergy testing and IgE serological testing are the two most common ways of detecting allergen sensitivity in dogs. Many clinicians prefer intradermal testing, as it has been used extensively for many decades and directly assesses the skin, the target organ of the disease, and its physiological response to allergens.<sup>4</sup> Intradermal testing is optimised by using allergens specific to the regional location of the patient and more accurate results are obtained when individual allergen extracts are used.<sup>5</sup> IgE serological assays involve ELISA based technologies to detect circulating IgE.<sup>6</sup> Despite some disagreement between intradermal and IgE serological tests,<sup>6</sup> studies have found no significant difference in the results of allergen-specific immunotherapy prescribed based on intradermal tests or IgE serological assays.<sup>7</sup>

Allergen-specific immunotherapy has been used to treat allergic dogs since the first reported successful treatment in 1941,<sup>8</sup> with varying degrees of success described in the literature. Currently, it is indicated in dogs with a definitive diagnosis of atopic dermatitis in which allergen sensitivity has been defined by intradermal or IgE serological tests.<sup>9</sup> It is currently the only therapy that can prevent further development of the disease, offer long-term remission and low ongoing treatment frequency to patients.<sup>9</sup>

The purpose of this study was to determine the prevalence of positive allergen reactions in dogs presenting to the Companion Animal Health Centre at the University of Adelaide's School of Animal and Veterinary Sciences in South Australia, from both intradermal and IgE serological tests. The clinical efficacy of subsequent allergen-specific immunotherapy was also evaluated.

## Materials and methods

### Data collection and study design

In this retrospective study, data were obtained from the Cornerstone practice management software used by the Companion Animal Health Centre at the University of Adelaide (South Australia). Records between 2011 and mid-2017 from dogs that underwent intradermal or IgE serological tests, and those that had been prescribed immunotherapy, were selected for analysis.

### Intradermal allergy test

Intradermal allergy tests were performed in 108 dogs (60 males and 48 females) at various ages ranging from 7 months to 9 years (median of 2). The intradermal allergy test comprised 60 allergens, made up of 3 mites, 5 insects, 18 trees, 15 grasses, 13 weeds,

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**Table 1.** Allergens included in the intradermal and IgE serological tests, and the prevalence of positive reactions

| Allergen                                          | Intradermal test prevalence (%) (n = 108) | IgE serological test prevalence (%) (n = 25) |
|---------------------------------------------------|-------------------------------------------|----------------------------------------------|
| <b>Mites</b>                                      |                                           |                                              |
| <i>Dermatophagoides farinae</i>                   | 29                                        | 24                                           |
| <i>Dermatophagoides pteronyssinus</i>             | 21                                        | 20                                           |
| <i>Tyrophagus putrescentiae</i>                   | 28                                        | 36                                           |
| <b>Insects</b>                                    |                                           |                                              |
| <i>Ctenocephalides felis</i>                      | 6                                         | 8                                            |
| Insect mix (house fly, moth, cockroach, mosquito) | 4                                         |                                              |
| Cockroach mix                                     | 6                                         | 8                                            |
| Mosquito                                          | 6                                         |                                              |
| Ant                                               | 13                                        | 16                                           |
| <b>Trees</b>                                      |                                           |                                              |
| Wattle                                            | 6                                         | 24                                           |
| Casuarina                                         | 7                                         | 28                                           |
| Eucalyptus                                        | 6                                         | 8                                            |
| Melaleuca                                         | 12                                        | 20                                           |
| Privet                                            | 6                                         | 16                                           |
| Birch                                             | 5                                         | 8                                            |
| Chinese elm                                       | 7                                         | 12                                           |
| Pine mix                                          | 4                                         | 16                                           |
| Red oak                                           | 4                                         | 16                                           |
| Olive                                             | 5                                         | 20                                           |
| Palm                                              | 7                                         |                                              |
| Peppercorn                                        | 10                                        |                                              |
| Black willow                                      | 10                                        | 8                                            |
| Plane tree                                        | 5                                         | 12                                           |
| Maple                                             | 16                                        | 24                                           |
| Silver poplar                                     | 7                                         | 16                                           |
| Liquidamber                                       | 5                                         | 12                                           |
| Cypress                                           | 5                                         | 4                                            |
| <b>Grasses</b>                                    |                                           |                                              |
| Cocksfoot grass                                   | 17                                        |                                              |
| Couch                                             | 13                                        | 32                                           |
| Kentucky bluegrass                                | 12                                        | 36                                           |
| Johnson grass                                     | 12                                        | 32                                           |
| Perennial ryegrass                                | 13                                        | 28                                           |
| Paspalum                                          | 8                                         | 28                                           |
| Canary grass                                      | 14                                        | 32                                           |
| Yorkshire fog                                     | 8                                         | 40                                           |
| Brome grass                                       | 17                                        | 32                                           |
| Sweet vernal                                      | 19                                        | 28                                           |
| Timothy grass                                     | 18                                        | 28                                           |
| Bent grass                                        | 10                                        | 28                                           |

**Table 1.** Continued

| Allergen                        | Intradermal test prevalence (%) (n = 108) | IgE serological test prevalence (%) (n = 25) |
|---------------------------------|-------------------------------------------|----------------------------------------------|
| Wheat                           | 7                                         |                                              |
| Oat                             | 8                                         |                                              |
| Alfalfa                         | 5                                         |                                              |
| <b>Weeds</b>                    |                                           |                                              |
| Kochia                          | 15                                        |                                              |
| Lamb's tongue                   | 10                                        | 16                                           |
| Fat hen                         | 15                                        | 20                                           |
| Ragweed                         | 10                                        | 24                                           |
| Rough pigweed                   | 16                                        | 28                                           |
| Sheep sorrel                    | 12                                        | 28                                           |
| Yellow dock                     | 25                                        | 36                                           |
| Red clover                      | 59                                        |                                              |
| Dandelion                       | 13                                        | 20                                           |
| Daisy                           | 13                                        | 12                                           |
| Mugwort                         | 6                                         | 12                                           |
| Tobacco                         | 4                                         |                                              |
| Dog fennel                      | 8                                         |                                              |
| Mustard weed                    |                                           | 20                                           |
| <b>Moulds</b>                   |                                           |                                              |
| <i>Alternaria tenuis</i>        | 4                                         | 4                                            |
| <i>Aspergillus</i> mix          | 5                                         | 0                                            |
| <i>Cladosporidium herbarum</i>  | 5                                         | 0                                            |
| <i>Penicillium</i> mix          | 5                                         | 0                                            |
| <b>Other</b>                    |                                           |                                              |
| Mixed feathers                  | 2                                         |                                              |
| <i>Malassezia pachydermatis</i> | 24                                        | 0                                            |

4 moulds, 1 feather and 1 yeast (Table 1). Allergens were imported from Greer (Lenoir, NC, USA) and purchased from Dermcare (Slacks Creek, Queensland, Australia). Histamine was used as a positive control at 0.0275 mg/mL and saline as a negative control. All allergens were tested at a concentration of 1000 PNU/mL except for the mites (1:2000 w/v); flea, ant and tobacco (1:1000 w/v); insect mix (400 PNU/mL); and cypress (1:400 w/v). Appropriate drug withdrawal times were applied before testing. Reactions were subjectively graded from 0 to 5 by comparing the size and erythema of the wheal to histamine, which was arbitrarily assigned a score of 4. Only immediate reactions (within 25 min of allergen injection) were recorded. Any reaction of 2 or greater was considered to be a positive reaction.

#### **IgE serological test**

IgE serological tests were performed in 25 dogs (14 males and 11 females) at various ages ranging from 8 months to 9 years (median of 2). Sixteen of these dogs had also undergone intradermal allergy testing. The remaining nine dogs only had the IgE serological test. IgE serological testing was undertaken in preference to intradermal testing

for several reasons, including owner preference, an inability to return to the clinic for the skin test, an aversion to clipping of the hair coat, concerns over sedation, or active skin disease over the testing site. IgE serological testing was performed using the Heska Allercept test (Heska, Loveland, CO, USA) and performed by Gribbles Veterinary Pathology (Glenside, South Australia, Australia). The test comprised 48 allergens made up of 6 mites/insects, 16 trees, 11 grasses, 10 weeds and 5 moulds/yeasts (Table 1). From 2011 until 2014, the reactions were graded using ELISA Absorbance units in which a result of 150 or greater was considered positive. From 2015 onwards, the test was measured using Heska IgE Receptor Binding Units and a result of 10 or greater is considered positive.

### Allergen-specific immunotherapy

Allergen-specific immunotherapy was prescribed to 37 dogs (17 males and 20 females) based on the results of the allergy tests. All of these dogs had undergone an intradermal test, but four dogs had also had an IgE serological test. No dogs were prescribed immunotherapy on the basis of an IgE test alone. Reasons for immunotherapy not being pursued included a negative allergy test, the typical slow rate of onset, the reported success rate, the cost of the treatment, the owner not wishing to inject their own dog, or a preference to try alternative therapies. Immunotherapy vials were formulated by Dermcare (Slacks Creek, Queensland, Australia) at a concentration of 15,000 PNU/mL. If 15 or fewer positive reactions were observed, all allergens were included in the treatment set. If more than 15 positive reactions were observed, 15 allergens were selected based on a subjective assessment of likely exposure, perceived relevance and potential cross reactivity between allergen groups. Immunotherapy was administered by subcutaneous injection by the owners following a standard schedule. Briefly, dogs were given gradually increasing doses of the allergen extract over a 22 day period, followed by administration of a full 1 mL dose every week for 3 doses, every 2 weeks for 3 doses and then every 3 weeks on a continuing basis. Dogs were evaluated after 3 and 9 months of treatment, and then at 8–12 month intervals (see A1 for dosage schedule).

### Assessment of treatment outcomes

The outcome of immunotherapy was determined using a global assessment of efficacy matrix (Table 2). This incorporated a collective assessment of multiple parameters, including pruritus score, lesion severity, requirement for additional medications and owner's and clinician's opinions. Due to case-to-case variation in the various parameters, the column descriptions were not applied rigorously, but were used as a guide to aid classification of a patient into one of four treatment responses (excellent, good, modest, none). In other words, a case had to meet the majority, but not all, of the criteria within a particular row to be classified as one of the treatment outcomes. However, ongoing requirement for anti-pruritic medications prevented a dog from being classified as a good or excellent response. This method of assessment was deemed most appropriate for a retrospective study of this type of treatment in which data have to be extracted from medical records and other modalities are typically used concurrently. Categorisation of each dog into one of the four treatment outcomes was performed independently by two of the authors (C. Han and P.B. Hill). In the case of any disagreements, the cases were further discussed until agreement was reached. Cases needed to have been on immunotherapy for at least 9 months to be judged as a success or a failure, unless the treatment had been stopped by the owner due to perceived lack of efficacy or the occurrence of adverse reactions prior to this. Analysis of the final outcome was undertaken in three different ways in relation to these withdrawals – as treatment failures, as potential treatment successes, or with withdrawn cases removed from the analysis altogether.

### Statistical analysis

Descriptive and analytical statistics were computed using Microsoft Excel and GraphPad prism. Prevalences of positive reactions were reported for allergy test results, and Fisher's exact test was used to compare age, sex and desexing status of dogs that received immunotherapy. A Kruskal Wallis test was used to compare the number of allergens used to treat dogs with different treatment outcomes.

**Table 2.** Global assessment of efficacy matrix: Outcomes of immunotherapy were classified into excellent, good, modest or failure based on pruritus level, pruritus score, skin lesions, requirement for concurrent medications and the owner and clinician's opinions of the dog's response to treatment

| Treatment outcome | Subjective pruritus level | Pruritus score | Skin lesions                       | Concurrent medications        | Owner's opinion   | Clinician's opinion                          |
|-------------------|---------------------------|----------------|------------------------------------|-------------------------------|-------------------|----------------------------------------------|
| Excellent         | None                      | 0–2            | None or very mild                  | No other medications          | Very satisfied    | Excellent response                           |
| Good              | Mild                      | 2–4            | Mild, but may have had some flares | Reduced but some still needed | Satisfied         | Good response                                |
| Modest            | Only slight reduction     | 4–6            | Moderate lesions                   | Ongoing for control of signs  | Unsure of benefit | Minimal response but may be worth continuing |
| Failure           | Unchanged                 | Unchanged      | Unchanged                          | Unchanged                     | No benefit        | Poor response and should stop treatment      |

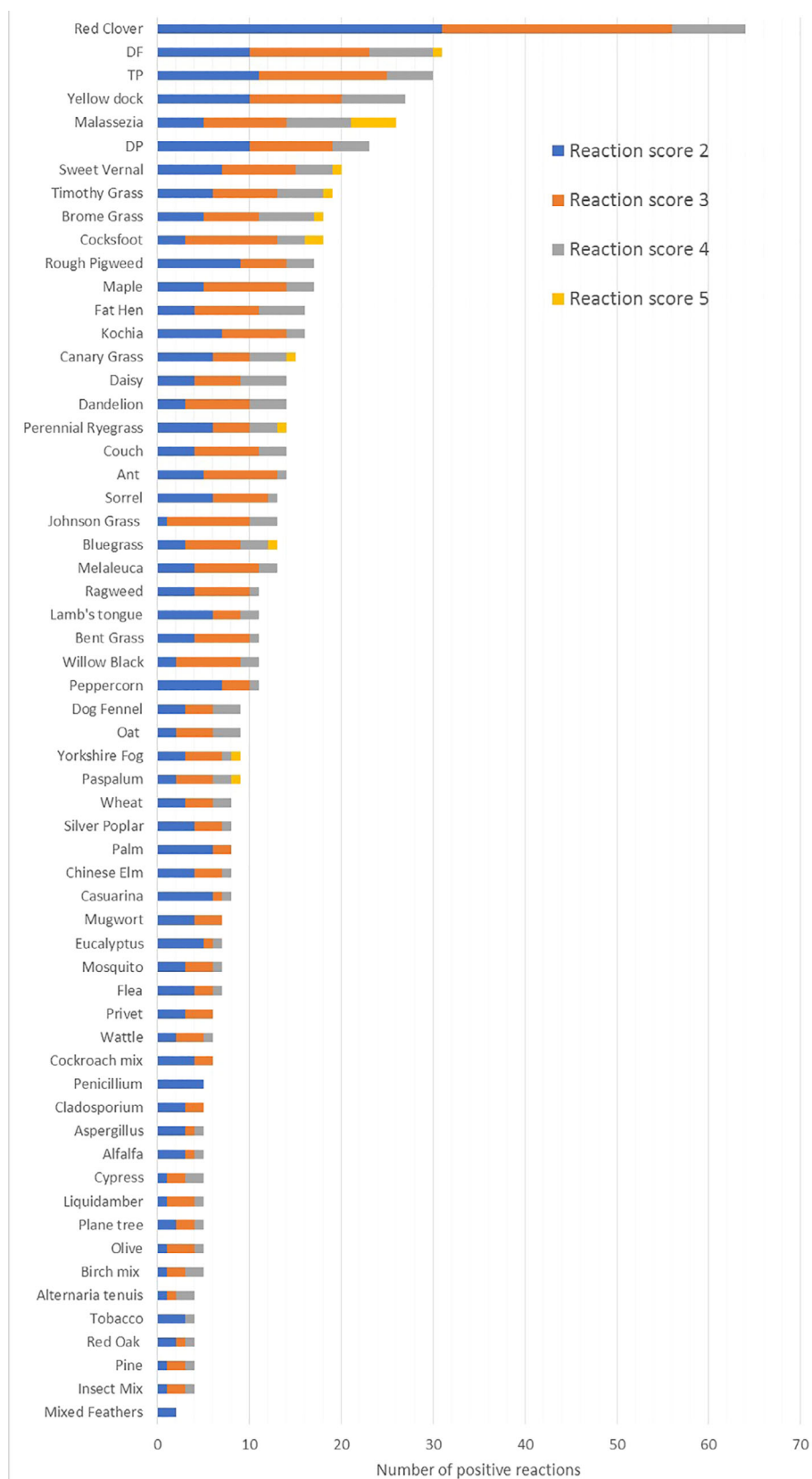


**Figure 1.** The number of positive reactions for each allergen in the 108 intradermal tests. The reactions were scored from 0–5 subjectively by comparison to the histamine reaction, which was arbitrarily assigned a score of 4. Blue represents a score of 2, orange a score of 3, grey a score of 4 and yellow a score of 5. DF, *Dermatophagoides farinae*; TP, *Tyrophagus putrescentiae*; DP, *Dermatophagoides pteronyssinus*.

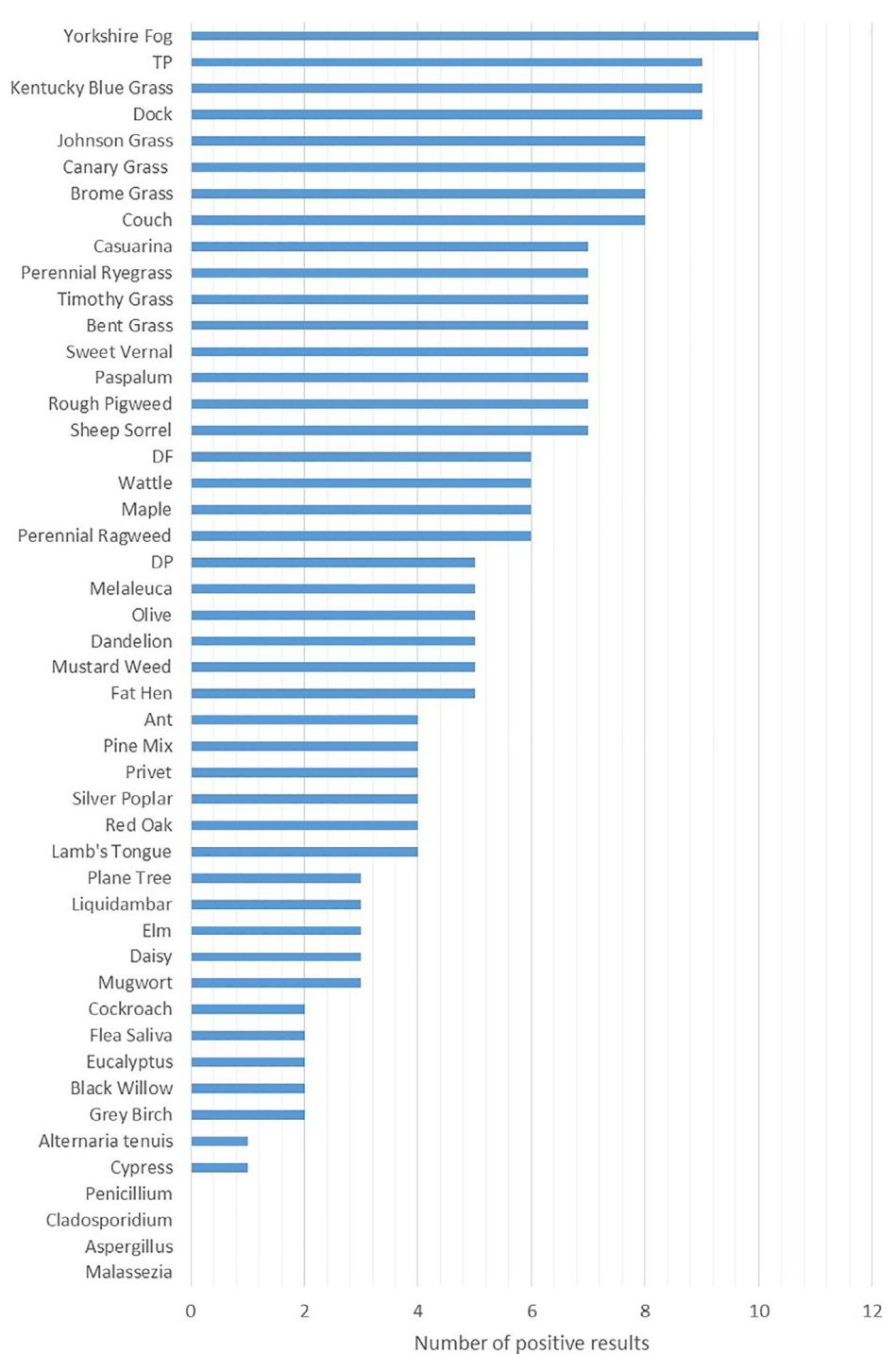


**Figure 2.** The number of positive reactions for each allergen in the 25 IgE serological tests. DF, *Dermatophagoides farinae*; TP, *Tyrophagus putrescentiae*; DP, *Dermatophagoides pteronyssinus*.

### Results

Of the 108 intradermal tests performed, 22 were negative and 86 were positive to at least one allergen (80%). Of the 25 IgE serological tests performed, 7 were negative and 18 were positive to at least one allergen (68%). The number of positive reactions seen for each allergen in the 86 positive intradermal tests and the 18 positive IgE serological tests is shown in Figures 1 and 2, respectively. To allow direct comparison, the

prevalence of positive reactions in the two tests is shown in Table 1. In the intradermal tests, the five most commonly reacting allergens were Red clover (positive in 64 tests, prevalence of 59%), *Dermatophagoides farinae* (n = 31, 29%), *Tyrophagus putrescentiae* (n = 30, 28%), Yellow dock (n = 27, 25%) and *Malassezia pachydermatis* (n = 26, 24%). Other allergens with a prevalence of positive reactions over 15% included *Dermatophagoides pteronyssinus*, sweet vernal grass, timothy grass, cocksfoot grass, brome grass, maple and rough pigweed. At least one

**Table 3. Outcome of allergen-specific immunotherapy in 34 dogs**

| Dog | Age | Sex | Breed               | Pruritus score | Skin lesions | Concurrent medications       | Clinician's opinion | Owner's opinion   | Comment                                                                                                 |
|-----|-----|-----|---------------------|----------------|--------------|------------------------------|---------------------|-------------------|---------------------------------------------------------------------------------------------------------|
| 1   | 2   | FS  | Great Dane          | 3              | Very mild    | None                         | Excellent           | Very satisfied    | No requirement for prednisolone and skin appeared virtually normal                                      |
| 2   | 5   | FS  | EBT                 | 2              | Very mild    | None                         | Excellent           | Very satisfied    | No requirement for Apoquel                                                                              |
| 3   | 1   | FS  | SBT                 | 2              | Very mild    | None                         | Excellent           | Very satisfied    | Skin appeared virtually normal, and no requirement for ongoing Apoquel                                  |
| 4   | 1   | FS  | Rottweiler          | 0              | None         | None                         | Excellent           | Very satisfied    | Dog appeared normal                                                                                     |
| 5   | 2   | MN  | Border Terrier      | 0              | Very mild    | None                         | Excellent           | Very satisfied    | Dog appeared normal                                                                                     |
| 6   | 1   | MN  | GSD                 | 3              | Very mild    | None                         | Excellent           | Very satisfied    | Dramatic improvement in pruritus scores and overall skin condition                                      |
| 7   | 4   | FS  | Shih Tzu            | 0              | None         | None                         | Excellent           | Very satisfied    | Owner considers the dog to be essentially normal                                                        |
| 8   | 1   | MN  | Brittany Spaniel    | 2–3            | None         | Occasional antihistamine     | Good                | Satisfied         | Skin appeared virtually normal, and no requirement for ongoing prednisolone                             |
| 9   | 3   | MN  | SBT                 | 3              | Mild         | Mometasone cream             | Good                | Satisfied         | Owner happy with pruritus level, but still some active dermatitis                                       |
| 10  | 1   | FS  | Lab                 | 2–4            | None         | Apoquel every 2–3 days       | Good                | Satisfied         | Overall improvement but still needs Apoquel every 2–3 days                                              |
| 11  | 2   | MN  | Bull Mastiff        | 3              | Mild         | Occasional antibiotics       | Good                | Satisfied         | AD controlled but still suffered intermittent folliculitis                                              |
| 12  | 4   | FS  | Border Terrier      | 2              | Mild         | Occasional ear drops         | Good                | Satisfied         | Occasional flare                                                                                        |
| 13  | 4   | M   | American Bulldog    | 1–5            | Moderate     | Mometasone cream/antibiotics | Modest              | Unsure of benefit | Waxing and waning with intermittent flares and recurrent furunculosis                                   |
| 14  | 5   | MN  | Dalmation           | 4              | Mild         | Apoquel                      | Modest              | Unsure of benefit | Owner thought there had been an improvement but still required daily Apoquel                            |
| 15  | 3   | FS  | Golden Retriever    | 2–4            | Moderate     | Prednisolone                 | Modest              | Unsure of benefit | Response was variable with multiple flares                                                              |
| 16  | 6   | FS  | Boxer               | 3–6            | Moderate     | Prednisolone/antibiotics     | Modest              | Unsure of benefit | Multiple flares requiring prednisolone                                                                  |
| 17  | 1   | FS  | BT                  | N/R            | Moderate     | Mometasone/prednisolone      | Modest              | Unsure of benefit | Owner considered good improvement but still active skin disease and recurrent Malassezia dermatitis     |
| 18  | 6   | FS  | Kelpie              | 4–6            | Moderate     | Cortavance                   | Modest              | Unsure of benefit | Owner considered skin disease better than before immunotherapy, but pruritus scores still high          |
| 19  | 4   | MN  | GSD                 | 5              | Mild         | Prednisolone                 | None                | No benefit        | No change in prednisolone requirement                                                                   |
| 20  | 1   | MN  | Doberman            | 5–6            | Moderate     | Prednisolone/Mometasone      | None                | No benefit        | Still required daily prednisolone                                                                       |
| 21  | 2   | FS  | Mastiff             | 3–6            | Mild         | Apoquel                      | None                | No benefit        | Still required daily Apoquel                                                                            |
| 22  | 2   | FS  | SBT                 | 2–3            | Moderate     | Apoquel                      | None                | No benefit        | Dog was never very pruritic but continued to get recurrent pyoderma. Eventually controlled with Apoquel |
| 23  | 2   | FS  | Boxer               | 5–7            | Mild         | Prednisolone/Apoquel         | None                | No benefit        | Relapsed when not on prednisolone or Apoquel                                                            |
| 24  | 1   | FS  | Boxer               | 3–6            | Moderate     | Apoquel                      | None                | No benefit        | Relapsed when not on Apoquel                                                                            |
| 25  | 1   | MN  | Irish Wolfhound     | 3–5            | Moderate     | Apoquel                      | None                | No benefit        | Relapsed when not on Apoquel                                                                            |
| 26  | 7   | MN  | Rhodesian Ridgeback | 5              | Moderate     | Prednisolone                 | None                | No benefit        | Owner discontinued immunotherapy with no follow up                                                      |
| 27  | 3   | MN  | Rottweiler          | 6.5            | Moderate     | Prednisolone                 | None                | No benefit        | Relapsed when not on daily prednisolone                                                                 |
| 28  | 2   | FS  | SBT                 | 3.5            | Mild         | Unknown                      | None                | No benefit        | Owner discontinued immunotherapy with no follow up                                                      |
| 29  | 4   | MN  | Poodle              | 2–3            | Mild         | Apoquel                      | None                | No benefit        | Relapsed when not on prednisolone or Apoquel                                                            |
| 30  | 1   | FS  | Labrador            | 4–8            | Moderate     | Prednisolone/Apoquel         | None                | No benefit        | Still required daily prednisolone or Apoquel                                                            |
| 31  | 4   | FS  | Lab                 | 6              | None         | Prednisolone                 | None                | No benefit        | Discontinued due to high pruritus score and prednisolone requirement                                    |
| 32  | 2   | MN  | Beagle              | 5–6            | Mild         | None                         | None                | No benefit        | Owner discontinued treatment due to high pruritus scores                                                |
| 33  | 6   | FS  | SBT                 | N/R            | Moderate     | Prednisolone                 | None                | No benefit        | Immunotherapy stopped due to swelling of neck after first few injections, with no follow up             |
| 34  | 6   | MN  | Lab                 | N/R            | Moderate     | Prednisolone                 | None                | No benefit        | Owner discontinued immunotherapy with no follow up                                                      |

The cases were assessed using the global assessment of efficacy matrix shown in Table 2. AD, atopic dermatitis; BT, bull terrier; EBT, english bull terrier; FS, female spayed; GSD, German shepherd dog; MN, male neutered; N/R, not recorded; SBT, staffordshire Bull terrier.

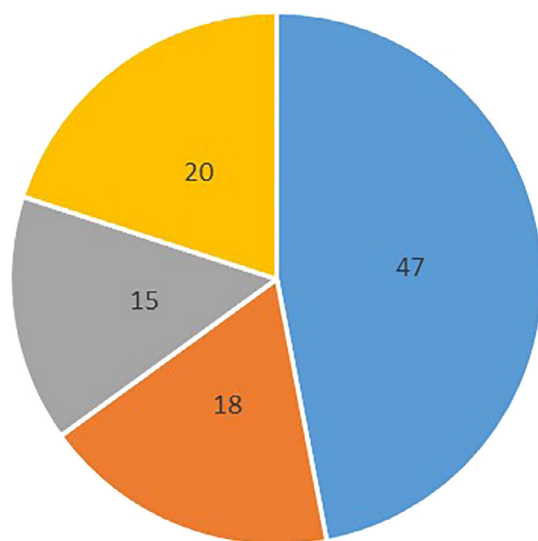
positive reaction was observed for all 60 allergens, albeit in some cases in a very small number of tests. The number of positive reactions in an individual dog ranged from 1 to 47 with a median of 5.

In the IgE serological tests, the four most commonly reacting allergens were Yorkshire fog grass (positive in 10 tests, 40%), *T. putrescentiae* (n = 9, 36%), Kentucky bluegrass (n = 9, 36%) and Yellow dock (n = 9,

36%). Twenty-eight other allergens had a prevalence of positive reactions over 15%. *M. pachydermatis*, *Penicillium*, *Aspergillus* and *Cladosporidium* had no positive results in any dogs tested. The number of positive reactions in an individual dog ranged from 1 to 40, with a median of 9.

Of the 37 dogs that were prescribed immunotherapy, three could not have their treatment efficacy assessed because they had not yet shown a beneficial response but had only been on treatment for 3 months at the time of the study. Four dogs failed to return for their first scheduled evaluation and the immunotherapy was discontinued. One owner discontinued the treatment due to an adverse reaction (swelling of the neck after the first few injections). For the data set of 34 cases, if the five withdrawals were classified as treatment failures, the outcome of immunotherapy was judged to be excellent in 20% of dogs, good in 15%, modest in 18% and a failure in 47% (Figure 3). If the five withdrawals had continued with the treatment, and their final outcomes were eventually good or excellent, the failure rate would have been 32% and the success rate would have been 50%. If these dogs were left out of the analysis altogether, there would have been a 38% failure rate and a 41% chance of a good or excellent outcome.

The number of allergens included in an immunotherapy vaccine had no significant effect on the outcome (Kruskal Wallis,  $P = 0.7$ ). 81% of dogs had their immunotherapy started at 4 years of age or younger, but there was no significant difference in efficacy between these dogs and those that were started later ( $P = 0.37$ ). Likewise, there were no significant differences in efficacy between males and females ( $P > 0.99$ ), and between entire and desexed dogs ( $P = 0.47$ ). However, these results should be interpreted with caution because the



■ Failure ■ Modest ■ Good ■ Excellent

**Figure 3.** The percentage of 34 dogs with outcomes of excellent, good, modest or failure in response to treatment with immunotherapy. Outcomes of immunotherapy were categorised based on a global assessment of efficacy matrix (Tables 2 and 3).

low numbers of patients may not have yielded sufficient power to demonstrate statistically significant differences.

## Discussion

In this study, household environmental mites, certain grass and weed pollens, and *M. pachydermatis* were shown to be the most relevant allergens in dogs with atopic dermatitis in South Australia. When comparing the two different testing methodologies (intradermal allergy testing and IgE serological assay), the prevalence of positive reactions to the environmental mites (*D. farinae*, *D. pteronyssinus* and *Tyrophagus putrescentiae*) were very similar in the two tests and in the region of 20%–30%. On a worldwide basis, dust mites and food storage mites are reported as very common allergens in canine atopic dermatitis with prevalence rates for positive reactions ranging from 14%–34% (South Eastern Australia),<sup>10</sup> 38% (Norway),<sup>11</sup> 42%–49% (UK),<sup>12</sup> 55% (France),<sup>13</sup> 54%–64% (Japan),<sup>14</sup> 61%–63% (Korea),<sup>15,16</sup> 53%–70% (Greece),<sup>17</sup> 53%–74% (Thailand),<sup>18</sup> and 71% in the USA.<sup>19</sup> The lower prevalence rates reported in this and the other Australian study may relate to the more rural lifestyle observed by dogs in some parts of the country.

The prevalence of positive reactions to grass, tree and weed pollen was generally higher in the IgE serological test compared to the intradermal test. In the intradermal test, tree pollens were typically positive in about 5%–10% of dogs, and grass and weed pollens were positive in about 10%–20% of dogs. In the IgE test, tree pollens were typically positive in 10%–25% of dogs with grasses and weeds yielding positive reactions in about 25%–30% of cases. The reason for this discrepancy is not known but it is accepted that intradermal testing and IgE serology do not correlate precisely.<sup>20–22</sup> However, whether the intradermal test is under-recognising pollen allergies (low sensitivity leading to false negative reactions) or the IgE assay is over-reporting pollen allergies (poor specificity leading to false positive reactions) is not known. One possibility for the lower rate of positive pollen reactions in the intradermal test could relate to the concentration of allergen used. Intradermal allergy testing is not a standardised procedure and different concentrations of allergens are reported by different authors. We used a concentration of 1000 PNU/mL for pollen allergens as recommended by the manufacturer, but other authors have reported concentrations as low as 500 PNU/mL.<sup>10</sup> Studies to assess irritant thresholds of allergens in normal dogs have reported tolerable concentrations of up to 8000 PNU/mL for certain allergens in one study<sup>23</sup> but the validity of this methodology was questioned in a subsequent publication.<sup>24</sup> However, It is possible that use of a higher allergen concentration may have yielded more positive pollen reactions. Yellow dock was the only pollen allergen that appeared in the top four reactions in both the intradermal test (fourth most prevalent reaction) and the IgE assay (second most prevalent reaction), confirming its importance as a major allergen in South Australia.

A major exception to these general findings was the high prevalence of positive reactions to Red clover in this study. 59% of dogs had a positive reaction to this allergen in the intradermal test, the highest in the set of 60. This allergen has not been widely reported in previous studies so direct comparison is not possible, but 10% of dogs

reacted to it in London and 5% in Edinburgh.<sup>12</sup> A number of possible explanations could account for this high rate of positivity. First, it could represent genuinely high rates of hypersensitivity to this allergen in South Australian dogs. Clovers are common in lawns and pastures<sup>25</sup> so there is ample opportunity for exposure. Also, Red clover is known not to cross-react with other weed allergens so mono-sensitisation would be more common than with other weeds.<sup>26</sup> Second, it could represent a high rate of false positive reactions in which an irritant component of the extract is able to elicit a wheal reaction in non-allergic dogs. This appears unlikely as nearly 40% of dogs did not show a reaction, and the phenomenon occurred over multiple batches of the allergen. A third possibility is that these are clinically irrelevant reactions that are still mediated by an IgE reaction but do not necessarily account for the dog's clinical signs. Unfortunately, this uncertainty cannot be resolved due to the lack of comparative data, and the absence of Red clover from the IgE serological test.

An important finding in this study that was not seen in most other studies of this type is the high prevalence of positive reactions to the commensal yeast *M. pachydermatis*. The immunological relevance of this yeast has only been recognised over the last 20 years<sup>27,28</sup> and it has only been available as a commercially available allergen for part of that time. *Malassezia* was not included as an allergen in most of the reported studies because they pre-dated its availability.<sup>10,19</sup> Despite this, it clearly represents a major allergen in dogs with atopic dermatitis, being positive in 24% of intradermal tests in this study. It also resulted in some of the strongest positive reactions, with many tests being scored as a 5 (meaning that the wheal size exceeded that of the positive control). The lack of positive reactions in the IgE serological assay is surprising, but this could simply be a quirk of the lower numbers tested. Other studies have reported positive serological reactions to the yeast in atopic dogs,<sup>15</sup> and immunotherapy against the organism has now been reported.<sup>29</sup>

The outcomes of allergen-specific immunotherapy reported in this study appear slightly less favourable than those previously reported. Only 35% of dogs were judged to have had a good or excellent response and 47% were considered failures. However, we did include 5 dogs in the failure group that had immunotherapy discontinued by their owners without veterinary input. It is possible that some of these might have improved if the treatment had been continued for longer. Our rationale for counting these dogs as failures was to allow a realistic overall impression of this type of treatment to be established. Despite initial counselling and guidance, some owners may discontinue this type of therapy because they perceive it is not working, is causing adverse effects, or is simply not appropriate for their needs. These owners are likely to consider the treatment a failure. In previous studies, success rates of 60%–75% are typically reported.<sup>30,31</sup> However, the lower success rate in our study can likely be explained by the group of cases referred to as 'modest' responses. The 18% of dogs in this category did appear to gain some degree of benefit from the treatment according to the owners, but it was not sufficient to permit other forms of treatment to be significantly reduced. Some of the apparent benefit could have been due to placebo effects because the only placebo controlled study to investigate the efficacy of immunotherapy reported that 20% of dogs receiving placebo injections improved by more than 50%.<sup>30</sup> In terms of outlining realistic expectations for an owner, the authors do not believe

that dogs in this group could be reasonably described as a meaningful success. Using this interpretation, we concluded that 65% of dogs did not gain great benefit from the treatment. However, other authors have interpreted these sub-optimal responses differently. For example, in one study,<sup>31</sup> the 20% of dogs that showed less than a 50% improvement were classified as having a 'moderate' improvement and were still judged a success, even though some of these dogs may have improved by only 10%. In another study, this group of dogs, representing 40% of the total, were classified as 'partial' responders (defined as being controlled but only with the use of concurrent systemic agents).<sup>32</sup> A further study defined a 'moderate' response as a minor improvement but the level of medication could not be decreased. Again, 20% of the dogs fell into this group. In each of these studies, a group of dogs with potentially very marginal improvements were being classified as having a moderate outcome, and hence a treatment success. Our results are most comparable to the study by Colombo,<sup>33</sup> which reported an excellent response in 22% of dogs, a measurable improvement in 22% of dogs, but no improvement in 56% of dogs.

In this study, we chose an end point at 9 months because this is when dogs came for re-evaluation after finishing their induction and initial course of maintenance vaccines. Many dermatologists assess the efficacy of immunotherapy after a 12 month period, but there is no scientific evidence as to why this should be the case. To the authors' knowledge, there is no reliable evidence that documents a cohort of dogs who improved dramatically between 9–12 months of treatment. There is also no sound immunological theory as to why an improvement would take 9 months to commence. As such, we do not believe that the outcomes of immunotherapy would have been significantly improved if the study had continued for 12 months. However, this cannot be proven one way or the other with the data generated in this study.

In conclusion, this study has revealed Red clover, environmental mites, Yellow dock, Yorkshire fog, Kentucky bluegrass and *M. pachydermatis* to be the most prevalent reactions in allergy tests in dogs in South Australia. Immunotherapy proved to be a successful treatment in 35% of dogs in which it was used.

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## APPENDIX

Table A1 Immunotherapy dosage schedule

|                      | Volume (mL) | Day          |
|----------------------|-------------|--------------|
| Vial 1 1500 PNU/mL   | 0.1         | 0            |
|                      | 0.2         | 2            |
|                      | 0.4         | 4            |
|                      | 0.6         | 6            |
|                      | 0.8         | 8            |
|                      | 1.0         | 10           |
|                      | 1.0         | 12           |
| Vial 2 15,000 PNU/mL | 0.2         | 14           |
|                      | 0.4         | 16           |
|                      | 0.6         | 18           |
|                      | 0.8         | 20           |
|                      | 1.0         | 22           |
|                      | 1.0         | +1 week × 3  |
|                      | 1.0         | +2 weeks × 3 |
|                      | 1.0         | +3 weeks     |

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