

Diagnosis of flea allergy dermatitis: comparison of intradermal testing with flea allergens and a FcεRI α-based IgE assay in response to flea control

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Abstract The objective of this study was to evaluate the accuracy of *in vivo* and *in vitro* tests in the diagnosis of flea allergy dermatitis in comparison with history, clinical signs and response to flea control. Intradermal testing using four different sources of flea allergens and FcεRIα-based immunoglobulin (Ig)E assays were performed in 15 flea-allergic dogs, 15 atopic dogs and 15 dogs infested with fleas but showing no clinical signs of skin disease. Sensitivity, specificity, negative predictive value, positive predictive value and accuracy were calculated for all five tests and results varied greatly. Sensitivity, specificity and overall accuracy were 27, 83 and 64%, respectively, for one extract (Isotec), 67, 90 and 82% for another extract (Greer), 93, 90 and 91% for flea saliva, 40, 90 and 73% for the recombinant Cte f 1 both produced by Heska Corp. and 87, 53 and 64% for a FcεRIα-based IgE assay. These results indicate that intradermal testing with flea extracts is more accurate in the diagnosis of flea allergy dermatitis than *in vitro* tests. Moreover, pure flea saliva used as a reagent for intradermal testing provided the best results in terms of sensitivity, specificity and overall accuracy although the Greer extract, a whole body flea extract, also allowed a good correlation between intradermal testing results and clinical approach to flea allergy dermatitis diagnosis.

Keywords: dogs, flea, IgE, intradermal.

INTRODUCTION

Flea allergy dermatitis (FAD) is one of the most common pruritic dermatoses in areas of the world where fleas are endemic. Its diagnosis relies on a thorough history and physical examination, elimination of other differential diagnoses, interpretation of the results of *in vivo* and/or *in vitro* allergy tests with flea allergens and response to appropriate antiflea treatment.

Suggestive historical data include the recurrence and/or presence of a pruritic dorsolumbar dermatitis in a young adult dog (> 1 year of age). Pruritus may or may not be seasonal, depending on the climate. One study¹ has shown a breed predisposition in chow-chows, Labrit Pyrenean shepherds, setters, fox terriers, Pekinese and spaniels, whereas other studies have failed to demonstrate this. Initially, corticosteroids allow a marked but temporary remission. The presence of fleas is some-

times reported but this only indicates infestation. In a French study, fleas were observed in only 65% of flea-allergic dogs. In 15% of these cases, neither fleas nor flea faeces were found.² In-contact animals, particularly cats, can be the source of infestation.

Erythema and papules observed in the dorsolumbar area and the inner and posterior thighs characterize the first stages of the disease. Sometimes these lesions become generalized. Episodes of pyotraumatic dermatitis may occur. Secondary lesions (excoriations, scaling, crusts, lichenification, hyperpigmentation, alopecia) appear gradually. Secondary infections such as superficial bacterial folliculitis or *Malassezia* dermatitis occur frequently.³

The differential diagnosis includes all pruritic dermatoses. The most common differentials are bacterial folliculitis, *Malassezia* dermatitis, scabies, trombiculosis, cheyletiellosis, pediculosis, adverse food reaction and atopic dermatitis (AD).

Allergy testing can be used to document sensitization of flea-allergic dogs to some flea antigens. However, a positive test does not necessarily mean that the animal tested has a clinical hypersensitivity at the time of testing. Results have always to be interpreted in the light of the history and clinical signs.

Intradermal testing (IDT) using flea extracts is considered by various authors to be an efficient and

Flea saliva and rCte f 1 were provided by Heska Corporation, 1613 Prospect Parkway, Fort Collins, CO 80525, USA.

Anti-flea products, except for Fontline® Top Spot and Advantage® Top Spot, were provided by Virbac Company, Treizieme rue, 06515 Carros cedex, France.

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determining element of a definitive diagnosis of FAD.^{2,4} Immediate reactions, read at 20 min, and/or delayed reactions, read at 24/48 h, can be observed after intradermal injection of flea allergens in flea-allergic dogs.^{3,5,6} The percentage of dogs showing one or both types of reaction may vary. Halliwell & Gorman⁶ found 60% of dogs with both immediate and delayed reactions, 25% of dogs showing only an immediate reaction and 15% of dogs showing only a delayed reaction (24 h). In contrast, Carlotti⁵ found 33% of dogs demonstrating only a delayed reaction. In this study, delayed reactions were read at 48 h. However, in these studies, only whole-body flea extracts were used. They were not biologically standardized and contained only a small percentage of salivary antigens.⁷ Such extracts are currently the only flea allergens commercially available for IDT. Purified fractions of these could be less active.⁸ Nevertheless, flea saliva and some of its fractions (purified or recombinant) gave rise to strong positive reactions when injected intradermally in sensitized dogs.⁹ Because of collection and cost problems, these flea salivary allergens are unavailable for in-practice use as reagents for IDT but they are used in a new *in vitro* test (Allercept®, Heska Corp., Fort Collins, CO, USA).⁹

The use of serological tests based on the detection by enzyme-linked immunosorbent assays (ELISA) of specific immunoglobulin (Ig)E or IgG for the diagnosis of FAD has also been a great source of debate. Sensitivity, specificity and reproducibility vary greatly, as does the quality of flea allergens used. Whatever the technique used, delayed reactions are missed.

Some authors have found high levels of IgE and IgG in allergic dogs when compared with control dogs,¹⁰ whereas others demonstrated the opposite.^{11,12} Moreover, cross-reactions with other insects may occur.¹³

The use of the high-affinity Fc epsilon receptor (FcεRIα) to detect antiflea saliva IgE in canine sera combined with highly purified flea salivary antigens (Allercept®) has been found to be a reliable test for FAD diagnosis.¹⁴

The aim of this study was to compare the results of five different tests (one serological test, Allercept® and IDT with two whole flea extracts, pure flea saliva and recombinant Cte f 1) with a clinical diagnosis of FAD based on history, clinical signs and response to appropriate antiflea therapy.

MATERIALS AND METHODS

Animal selection

Dogs were recruited at a dermatological referral centre and in two general veterinary practices around Bordeaux (France) over a period of 1 year. Most cases were seen during summer 2001.

Three groups of dogs were defined. Group 1 included dogs with FAD but no or only minor signs of AD, whereas group 2 included atopic dogs as defined by Prélard *et al.*¹⁵ Group 3 included dogs infested with fleas but showing no sign of skin disease.

Diagnosis of FAD in group 1 was based on historical criteria (recurrent pruritic dermatitis involving the dorsolumbar area, recent flea infestation) and clinical signs: pruritus, presence of at least one primary lesion (erythema and/or papules) and two secondary lesions (excoriations and/or crusting and/or alopecia and/or scaling and/or lichenification and/or hyperpigmentation) in the dorsolumbar area and possibly in other areas.¹⁶ Dogs with major clinical signs of AD (e.g. facial dermatitis, otitis externa, pododermatitis, carpal and/or tarsal dermatitis) were excluded from this group. At this point, as FAD was our major differential diagnosis, we did not perform food trials in group 1 dogs. On the day of inclusion, clinical signs were evaluated using the Scoring System for Canine Flea Allergy Dermatitis (SSCFAD) which includes an assessment of clinical lesions (Lesional Index for Canine Flea Allergy Dermatitis, LICFAD, see Table 1) and pruritus (Pruritus Index for Canine Flea Allergy Dermatitis, PICFAD, see Tables 1–3). As there was no validated scoring system for FAD at the time the study was performed, we decided to use the SSCFAD, which had been developed previously by Pierre Jasmin and Didier-Noël Carlotti for an unpublished study on the treatment of FAD. We have found that this system provides a convenient and accurate method to evaluate response to therapy in FAD. For a dog to be included, a LICFAD of at least 180 and a PICFAD of at least 20 (as determined by the investigator) at day 0 were necessary. This cut-off point was decided arbitrarily.

Response to appropriate antiflea therapy allowed a definitive diagnosis of FAD in this study. Dogs treated previously for fleas were excluded if the duration of effect of the product used had not expired (according to the manufacturer). Owners were asked to treat their flea-allergic dogs once a week as follows. A nonmedicated shampoo (Sebocalm®, Virbac Co., Carros, France) was applied followed by spraying of a humectant (Humilac®, Virbac) followed by spraying of a permethrin pump-spray (Defendog®, Virbac) or Duowin® (permethrin and pyriproxyfen, Virbac) as recommended by the manufacturer (i.e. 5 mL/kg). In-contact dogs had to be treated with the same permethrin pump-spray, at a minimum frequency of once every 2 weeks. In-contact cats had to be treated with fipronil spray or spot-on (Frontline®, 7.5 mg/kg, Merial, Lyon, France) or with imidacloprid spot-on (Advantage®, 0.4 mL for a cat weighing < 4 kg or 0.8 mL for a cat weighing > 4 kg, Bayer, Puteaux, France) once a month. The environment was also treated on two occasions, 3 weeks apart, with a pump-spray containing both an adulticide and an insect growth regulator (Permethrin 0.5 g/100 mL, Piperonyl butoxide 1 g/100 mL and Pyriproxyfen 5 mg/100 mL, Parastop®, Virbac). No symptomatic treatment of the pruritus was permitted during the study. Decrease in the LICFAD and PICFAD of at least 25% at day 15 and at least 75% at day 30 were required for any dog to be included in the study. The clinician assessing response to flea control was aware of IDT results at the time of the control visits.

Table 1. Scoring System for canine Flea Allergy Dermatitis (SSCFAD). The scores are based on evaluation of pruritus and lesion severity and distribution. The maximum possible lesion score was 360 and the maximum possible pruritus score was 40. Dogs had to have scores at least 180 and 20 to be included in the FAD group

Anatomic area	Site	Erythema	Papules	Excoriation	Alopecia	KSD	Lichenification	Manifestation	Pruritus
<i>Lesional Index for Canine Flea Allergy Dermatitis (LICFAD)</i>									
Dorso-lumbar area	Dorsal neck, back, lumbar, tail	0–10	0–10	0–10	0–10	0–10	0–10	–	–
Lateral area	Trunk, flank (left and right)	0–10	0–10	0–10	0–10	0–10	0–10	–	–
Hind limb area	Tarsus, knee and thigh (left and right)	0–10	0–10	0–10	0–10	0–10	0–10	–	–
Ano-genital area	Anus, perineum, genital area	0–10	0–10	0–10	0–10	0–10	0–10	–	–
Front-ventral area	Axilla (left and right), sternum	0–10	0–10	0–10	0–10	0–10	0–10	–	–
Hind-ventral area	Inguinal (left and right), abdomen	0–10	0–10	0–10	0–10	0–10	0–10	–	–
Parameters total score		/60	/60	/60	/60	/60	/60		
<i>Pruritus Index for Canine Flea Allergy Dermatitis (PICFAD)</i>									
Dorso-lumbar area		–	–	–	–	–	–	rubbing/licking/chewing scratching/rubbing/licking	0–10
Lateral and ventral areas		–	–	–	–	–	–		0–10
(trunk, axillae and abdomen)		–	–	–	–	–	–	licking/chewing rubbing/licking/chewing	0–10
Hind limbs		–	–	–	–	–	–		0–10
Ano-genital area		–	–	–	–	–	–		0–10
Pruritus total score									/40

Scale 0 = none; 10 = severe. KSD: kerato-seborrheic disorder.

Table 2. Pruritus grading scale for PICFAD (including frequency and intensity) with a 0–10 score

Frequency of pruritus	Intensity of pruritus			
	Low	Moderate	Important	Severe
None				
Occasional	1	2	3	4
Quite frequent	3	4	5	6
Frequent	5	6	7	8
Quasi permanent	7	8	9	10

Failure to adhere to the treatment protocol, particularly in relation to the use of antipruritic drugs (i.e. glucocorticoids, antihistamines and medicated topicals) or failure of response to therapy as defined above, led to dogs being withdrawn from the study.

Diagnosis of AD in group 2 was based on Prélard *et al.*'s criteria¹⁵ (Table 4). For the purpose of the study, dogs included in this group had to be free of fleas for more than 3 months (no recent history of flea infestation, no flea or flea faeces present on the day of inclusion after a 10-min combing with a flea comb). Presence of lesions in the dorsolumbar area suggesting FAD as defined above, was not allowed for group 2 dogs. Adverse food reactions had to be ruled out by a home-cooked elimination diet involving a novel source of protein and carbohydrate for at least 6 weeks.⁴

For these two groups, secondary bacterial folliculitis and *Malassezia* dermatitis, if present, had to be cleared with specific treatment prior to inclusion in the study. Other ectoparasitic infestations (scabies, cheyletiellosis, trombiculosis, pediculosis, ear mite infestation or demodicosis) were ruled out either by microscopical examination of skin scrapings, brushings and tape strips or by trial acaricidal therapy (ivermectin; Ivomec^R, Merial, 0.3 mg/kg subcutaneously, given on two occasions with a 14-day interval). Antipruritic drugs were withheld prior to inclusion according to recommendations in the literature (systemic antihistamines 10 days, topical corticosteroids 2–3 weeks, systemic corticosteroids at least 1 month).⁴

Dogs recruited to group 3 were presented for routine procedures such as vaccination, identification, spaying. Dogs in group 3 had no history of skin disease but owners reported the intermittent presence of fleas. Fleas or flea faeces had to be demonstrated on the day of inclusion by using a flea comb for up to 10 min. Black specks suspected of being flea faeces were placed on wet blotting paper. If red streaking was observed, the presence of flea faeces was confirmed.

IDT with flea antigens

Allergens used Isotec Laboratories (St Quentin en Yvelines, France) provided us with a whole-body flea extract at a concentration of 1:1000 (w/v).

The aqueous extract of whole-body *Ctenocephalides* spp. manufactured by Greer Laboratories (NC, USA) was diluted in saline to a concentration of 1:1000 (w/v). Aliquots were stored at 6 °C.

Table 3. Definitions for pruritus scoring using the PICFAD

	Definitions
<i>Frequency of pruritus</i>	
Occasional	Less than once to once a day
Quite frequent	A few to several times a day but the animal is sometimes seen not scratching
Frequent	At least once during each period the animal is seen
Quasi permanent	Several times during each period the animal is seen ('more time spent scratching than not')
<i>Intensity of pruritus</i>	
Low	The animal shows low attention when scratching and/or scratches for very short periods (a few seconds)
Moderate	The animal is concentrating when scratching and/or scratches for short periods (several seconds)
Important	Very nervous animal when scratching and/or scratches for quite long periods (1 to a few minutes)
Severe	The animal may be aggressive when scratching and/or cannot stop when asked and/or scratches for long periods (several minutes)

Table 4. Clinical diagnosis of atopic dermatitis in the dog according to Prélard *et al.*'s criteria¹⁵

<i>At least 3 major criteria</i>	
Onset of clinical signs between 6 months and 3 years of age	
Corticosteroid responsive pruritus	
Anterior interdigital erythematous bilateral pododermatitis	
Erythema of the medial pinnae	
Cheilitis	
<i>Suggestive criteria (nonvalidated)</i>	
Breed predilection or familial predisposition	
Dull coat	
Lesions on palmar/plantar carpus/tarsus	
Lesions of the fold of the stifle	
Chronic or recurrent dermatitis lasting for more than 2 years	
Acral lick dermatitis	
Hyperhidrosis	
History of urticaria or angioedema	
Seasonal worsening of signs	
Worsening when the dog walks in grass	
Variation of signs according to dog's surroundings	

Flea saliva and a recombinant flea saliva allergen (Cte f 1) were supplied by Heska Corp. Flea saliva was diluted in saline to a concentration of 20 µg/mL (i.e. 1 µg per 0.05 mL IDT injection) and aliquots were stored at 6 °C. Cte f 1 was diluted in saline to a concentration of 20 µg/mL (i.e. 1 µg per IDT injection). Aliquots were stored at -18 °C.

Two controls were also used: a positive control (histamine phosphate 0.01%) and a negative control (phenolated physiological diluent).

Standard protocol IDT were conducted with the owner's consent in accordance with a standardized and commonly used protocol, without general anaesthesia. Dogs were placed in lateral recumbency and clipped carefully over the thorax. After cleaning the area with ether, injection sites were marked using a felt-tip pen. Each solution (0.05 mL) was injected strictly intradermally in a standard order, using sterile 29G insulin syringes with fixed needles (0.33 mm).

Assessment of reactions Reactions were first read after 20 min, in the dark with the aid of an oblique light source. A raised erythematous wheal was considered a positive reaction. If erythema was absent, the result was considered negative, even if a visible small wheal was visible. The greatest diameter of each reaction was

measured precisely using a ruler provided by the allergen suppliers. To be considered positive, the diameter of the wheal at the suspected allergen injection site (A) had to be greater than or equal to the mean of the wheal diameters at the histamine (H) and solvent (C) control sites.

When the reaction to flea extract at 20 min was negative, a second measurement was made at 48 h. This interval was considered optimal because the immediate reaction can sometimes persist for up to 24 h. Moreover, in a delayed reaction, maximal development of a cutaneous lesion provoked by intradermal injection of antigen in a sensitive individual occurs between 12 and 72 h.² The delayed reaction to flea extract appears as skin thickening (detected by palpation of a skin fold) or as a papule, both of which can be encrusted. The delayed reaction was not always evaluated in dogs showing an immediate reaction, as this is sufficient to confirm hypersensitivity to the extract(s).² Delayed reactions were checked by the investigator as often as possible. When it was impossible for the investigator to check the reactions, owners were instructed how to assess these delayed reactions 48 h after skin testing. A written document summarizing the date of checking and of the range of lesions they might observe was given to them. Owners were asked to report the results scrupulously to the investigator.

In vitro testing

A blood sample was taken from each dog ≈ 30 min after IDT and centrifuged. The serum sample was stored at -18 °C until it was sent to Heska Corp. for Allercept® testing. This *in vitro* assay is based on the use of the IgE-specific receptor FcεRIα. An optical density > 150 EA units in the Allercept® was considered to be a positive result for this test (cut-off determined by Heska Corp.).

Sensitivity, specificity, positive predictive value, negative predictive value and accuracy determination

For each allergenic extract and for the *in vitro* test, calculations of sensitivity, specificity, positive predictive value, negative predictive value and accuracy were performed as follows.

- Sensitivity: True positive/true positive + false negative.

- Specificity: True negative/true negative + false positive.
- Positive predictive value: True positive/true positive + false positive.
- Negative predictive value: true negative/true negative + false negative.
- Accuracy: true positive + true negative/total.

Group 1 dogs were true flea-allergic dogs (a positive reaction in this group was a true positive and a negative reaction was a false negative). Group 2 dogs were atopic but not flea allergic. Group 3 dogs were normal dogs with a flea infestation. In groups 2 and 3, a positive reaction was a false positive and a negative reaction was a true negative.

Calculations were performed with the three groups all together (group 1 compared with groups 2 and 3). Inclusion of atopic dogs (group 2) in the control group can be justified by the fact that atopic dogs are prone to develop hypersensitivity to flea allergens, possibly showing no clinical signs (subclinical sensitization).^{1,3,4,6,17}

RESULTS

Epidemiological data

Forty-five dogs of varying age, sex and breed were included in the study (15 in each group). Owing to the low number of cases, no breed predilection was calculated.

Five entire males, nine entire females and one spayed female were included in group 1. Age at diagnosis varied between 1 and 13 years (mean: 6.4 years).

Five entire males, seven entire females and three spayed females were studied in group 2. Age at diagnosis varied between 1 and 9 years (mean: 4.6 years).

Nine entire males and six entire females were included in group 3. Their age varied between 1.5 and 7 years (mean: 3.5 years).

Clinical data

The scores achieved for each group 1 dog at days 0, 15 and 30 are presented in Fig. 1 for the LICFAD and in Fig. 2 for the PICFAD. Most lesions and pruritus were localized in classical FAD areas.

Interpretation of the IDT and Allercept® results

Interpretation of the IDT was possible in all cases. No adverse reactions associated with the IDT were seen.

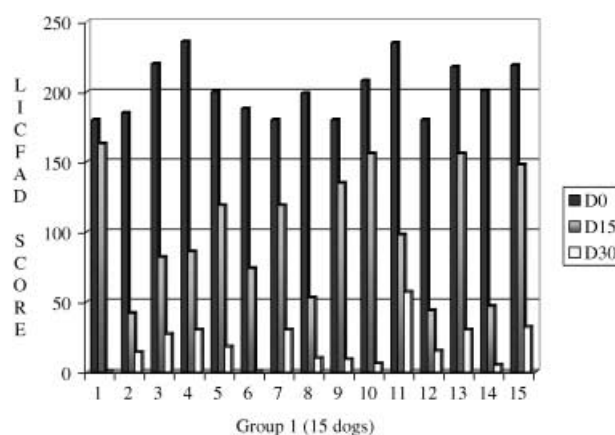


Figure 1. Evolution of lesional score (LICFAD) for each dog during treatment.

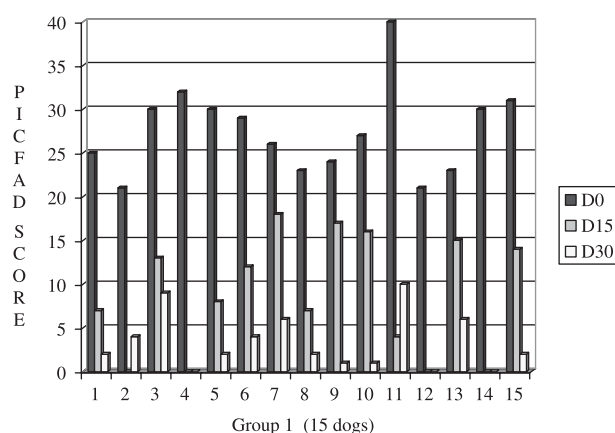


Figure 2. Evolution of pruritus score (PICFAD) for each dog during treatment.

The results of immediate IDT reactions are shown for the three groups of dogs in Table 5.

Delayed reactions were as follows. In group 1, no delayed reaction was seen by any of the seven owners who checked for this. Positive delayed reactions were seen by the investigator in two of seven dogs: one dog had a positive delayed reaction to Greer extract and to Cte f 1, one dog was found to have a positive delayed reaction to Greer extract, flea saliva and Cte f 1. The two dogs with positive reactions at 48 h had already been found to be positive at 20 min. In the remaining five dogs, no delayed reaction was seen by the investigator. The presence of a delayed reaction was not checked for in one dog (because there had been a positive reaction at 20 min to all the allergens).

In group 2, delayed reactions were checked by the investigator in seven dogs and by the owner in eight

Table 5. Number of immediate reactions (% of dogs), read 20 min after intradermal injection of each allergenic extract, in the three groups of dogs

	Group 1 Flea-allergic dogs (n = 15)		Group 2 Atopic dogs (n = 15)		Group 3 Normal dogs with flea infestation (n = 15)	
	True positive	False negative	True negative	False positive	True negative	False positive
Isotec extract	4 (27)	11 (73)	11 (73)	4 (27)	14 (94)	1 (6)
Greer extract	10 (67)	5 (33)	15 (100)	0 (0)	14 (94)	1 (6)
Flea saliva	14 (94)	1 (6)	12 (80)	3 (20)	15 (100)	0 (0)
Cte fl	6 (40)	9 (60)	13 (87)	2 (13)	15 (100)	0 (0)

Table 6. Combined results of immediate and delayed reactions following intradermal injection of flea allergens

	Group 1 Flea-allergic dogs (<i>n</i> = 15)		Group 2 Atopic dogs (<i>n</i> = 15)		Group 3 Normal dogs with flea infestation (<i>n</i> = 15)	
	True positive	False negative	True negative	False positive	True negative	False positive
Isotec extract	4 (27)	11 (73)	11 (73)	4 (27)	14 (94)	1 (6)
Greer extract	10 (67)	5 (33)	14 (94)	1 (6)	13 (87)	2 (13)
Flea saliva	14 (94)	1 (6)	12 (80)	3 (20)	15 (100)	0 (0)
Cte fl	6 (40)	9 (60)	13 (87)	2 (13)	14 (94)	1 (6)
Allercept	13 (87)	2 (13)	9 (60)	6 (40)	7 (47)	8 (53)

Table 7. Calculation of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy. Group 1 dogs had flea allergy dermatitis. In the control group, atopic dogs (group 2) and flea-infested dogs without skin disease (group 3) were considered together

Group 1 vs. 2 + 3	Sensitivity	Specificity	PPV	NPV	Accuracy
Isotec extract	0.27	0.83	0.44	0.67	0.64
Greer Extract	0.67	0.9	0.77	0.84	0.82
Flea saliva	0.93	0.90	0.82	0.96	0.91
Cte fl	0.4	0.9	0.67	0.75	0.73
Allercept	0.87	0.53	0.48	0.89	0.64

dogs. No delayed reaction could be observed in 14 dogs (94% of cases). The Greer extract elicited a false-positive delayed reaction, found by the investigator in one dog (6% of cases). In this dog, all immediate reactions had been negative.

In group 3, delayed reactions were checked for by the investigator in all the 15 dogs. No delayed reaction could be observed in 13 dogs (87% of the cases). The Greer extract and Cte f l elicited a false-positive delayed reaction in one dog each (6% of the cases), this was assessed by the investigator. In these two dogs, none of the immediate reactions was positive.

The results with the Allercept® were as follows. In group 1, this test was positive in thirteen dogs (87%) and negative in two (13%). In group 2, Allercept® with flea saliva was positive in six dogs (40%) and negative in nine (60%). Finally, in group 3, Allercept® was positive in seven dogs (47%) and negative in eight (53%).

Table 6 summarizes these results, taking into account immediate and delayed reactions together.

Sensitivity, specificity, positive predictive value, negative predictive value and accuracy determination

These parameters were calculated for all the dogs together (groups 1, 2 and 3). The results are shown in Table 7.

DISCUSSION

In this study, we found it difficult to recruit strictly flea-allergic dogs without clinical signs of AD. Often dogs with a clinical presentation suggesting mainly FAD also exhibited some pruritus and lesions in areas usually associated with AD. These dogs could not, therefore, be included in the study. This is in accordance with the results of a study conducted in Aquitaine in the mid-

1990s which found that FAD is rarely encountered alone.¹ In another study, only 76% of dogs with FAD exhibited dorsolumbar region involvement. Facial pruritus and/or lesions were noted in 34% of these dogs but feet were involved in only 1.2%. However, the dorsolumbar region was involved in 39% of atopic dogs.¹⁸ Whether these signs were due to an adverse food reaction and/or AD or to FAD could be evaluated, for example, using appropriate antiflea therapy.

In our study, three dogs from group 1 still exhibited very mild clinical signs consistent with AD (licking of the feet, rubbing of the face) after 1 month of strict antiflea treatment. As permethrin either used as a spot-on or spray has already been found to have a knock-down effect and thus be effective in FAD treatment, even in very hypersensitive dogs^{19,20} it is likely that these dogs showed a mild degree of adverse food reaction and/or AD. Indeed, one study showed that 80% of the flea-allergic dogs were also sensitized to aero-allergens.¹ By contrast, in the same study, 33% of the atopic dogs had a positive IDT to flea.¹

We found more false-positive reactions against all flea allergens taken together in the group of atopic dogs (10 false-positive reactions in group 2) than in the group of dogs with flea infestation (4 false-positive reactions in group 3). This probably reflects the fact that atopic status may predispose dogs to FAD.^{1,6,17} It may also represent hyperirritable skin and/or cross-reaction with other allergens. In this study, all 10 atopic dogs with false-positive results to flea extracts, showed positive reactions to *Dermatophagoïdes farinae* and other mites except one dog, which had a positive result with the Allercept® only. Three of them also had a positive reaction to cockroach.

The occurrence of false-positive reactions to flea extracts in normal dogs has meant that the IDT in the diagnosis of FAD has always been controversial, some authors not using it because of its poor specificity.^{21,22} In a flea-rich environment (e.g. southern Florida, USA), 24% of clinically normal dogs (21 of 86) showed positive immediate skin test reactivity to a Greer flea extract. This was not predictive of the future development of FAD. Two years later, only 2 of 86 dogs (2.5%) had developed clinical signs of FAD.²² However, in a flea-scarce environment (Oslo, Norway), only 2 of 40 dogs with clinically diagnosed AD and none of 33 normal dogs or dogs with diseases other than AD had a positive immediate reaction against flea. In that area, prevalence for positive IDT against flea was 2.7%.²⁴

Positive reactions against flea allergens in atopic and clinically normal dogs in a flea-rich environment might represent truly false-positive (irritant) reactions, sub-clinical hypersensitivity²³ or cross-reactivity to other insect antigens.¹³

Furthermore, it must be emphasized that not all flea allergenic extracts used for IDT in our study had the same diagnostic value, particularly in terms of specificity: we compared four of them and results varied greatly. When we considered the results of group 1 with those of groups 2 and 3, the Isotec extract had the lowest sensitivity (27%), specificity (83%) and overall accuracy (64%). These results are in contrast to a previous study (1985), in which Carlotti found IDT with this allergenic extract to be reliable as an aid in the diagnosis of FAD (4% false-negative results and no false-positive results).⁵ Whole-body flea extracts are not standardized and variations in their composition and allergenicity may occur;^{25,26} this may explain this discrepancy.

With the Greer extract, IDT sensitivity was 67%, specificity 90% and overall accuracy 82%. These results are slightly lower than those published previously. In one study, when the 'half-way point' grading system was used here, 100% of the flea-hypersensitive and 67% of the normal dogs had a positive intradermal reaction with this extract.⁸ However, the most accurate grading system was found by these authors to be the one which considered a reaction to be positive when its diameter was at least 5 mm larger than the negative control's diameter. With this method of scoring, 94% of the flea allergic and none of the normal dogs had a positive reaction with the Greer extract.⁸

Pure flea saliva from Heska Corp. provided the most accurate results in the IDT, comparing them with the definitive diagnosis of FAD based on history, clinical signs and response to antiflea treatment. Sensitivity was 93%, specificity 90% and overall accuracy 91% in this study. This seems to support the suggestion that allergens involved in flea allergy dermatitis should be found in flea saliva.^{6,9,25} In one study, when antibodies from flea-infested mice were placed on fixed sections of whole fleas, the antibodies bound only to salivary gland structures.²⁷ The fact that flea saliva only represents 0.5% of the proteins in whole-body flea extracts might somehow explain why IDT with whole-body flea extracts has been associated with variable results.⁷ However, some authors believe that some nonsalivary antigens might exist and also be important in the pathogenesis of FAD. They would be missed with the pure flea saliva but not with whole-body extract.⁴ This seems to us not to be the case, as using pure flea saliva in our study enabled a more precise diagnosis to be made in terms of not only specificity, but also sensitivity.

Cte f 1 is an 18 kDa protein isolated from flea saliva and is a major allergen.²⁸ In our hands, its use as a reagent for IDT in the diagnosis of FAD did not give as accurate results as was seen with pure flea saliva, particularly in relation to sensitivity. When comparing group 1 with groups 2 and 3, sensitivity was 40%, specificity was 90% and overall accuracy was 73%.

Other studies either with salivary extract¹¹ or whole-body extract^{8,29} failed to isolate the same protein as a major allergen. Greene *et al.* found at least 15 different flea allergens, with molecular masses ranging from 14 to 150 kDa, able to bind IgE from flea-allergic dogs' sera. Three of them with apparent molecular masses of 25, 40 and 58 kDa, were recognized by 40% of these sera and not by the sera of normal dogs.²⁹ In Stolper & Opdebeeck's study, most of the allergenic molecules responsible for positive intradermal reactions in flea-allergic dogs had a molecular mass < 30 kDa, and one of them could elicit a positive intradermal reaction in 59% of the flea-allergic dogs.⁸ A more recent study showed that two proteins isolated from flea saliva with apparent molecular masses of 8–12 kDa and 40 kDa, respectively, might be important in FAD.¹¹ Dilution and storage conditions of Cte f 1 might also affect its allergenicity. Having made the correct dilution, we stored the protein at –18 °C in aliquots containing enough Cte f 1 to perform IDT in six dogs. Perhaps storing nondiluted Cte f 1 would have helped to preserve its properties.

Taken alone, reading delayed reactions was not a very sensitive method of diagnosing FAD as most dogs in group 1 (FAD) were negative. One could argue that half of the delayed reactions were checked by the owner after instruction by the investigator and that the experienced eye of the investigator might have detected subtle delayed reactions. This is unlikely. In addition, this would not have changed the results of IDT with flea saliva as 14 of 15 dogs had a positive immediate reaction. The majority of delayed reactions were negative in the groups of atopic and control dogs (3 false positives of 30). In group 3, delayed reactions were looked for by the investigator. None was present. In group 2, only seven dogs were seen by the investigator, because owners lived too far from the clinic. It seems unlikely, however, that many of these atopic dogs had a positive delayed reaction to flea extracts, missed by the owners.

In this study, IDT with flea extracts was more accurate than *in vitro* testing for the diagnosis of FAD, even when the most recent *in vitro* test was used. In a previous study, it was shown that the Allercept® combined greater specificity (by using the FcεRIα to detect IgE) and greater sensitivity (by using flea saliva and recombinant Cte f 1 as allergens) than the other *in vitro* assays.¹⁴ In that study, it was found to give excellent results: when compared with IDT results with flea saliva, sensitivity was 78%, specificity was 91% and accuracy was 88%.¹⁴ In our study, where results of one test were compared with clinical diagnosis and therapeutic outcome, 13 dogs (of 15 in group 1) had a true-positive reaction and 2 had a false-negative result. However, 14 dogs (of 30 in groups 2 and 3) were found to have a false-positive result with the Allercept®. A negative result with this test made the diagnosis of FAD unlikely but a positive result could not allow any conclusion to be made. If the cut-off point was raised (e.g. to 200 EA units), specificity would not increase very much but sensitivity would decrease very quickly: two dogs in

group 1 had an optical density between 150 and 200 EA units and would have been considered false negative if the cut-off point had been raised to 200 EA units.

One would have expected the two dogs that gave false-negative results with the Allercept® to have only delayed reactions with IDT. This is because the Allercept®, an IgE assay, cannot detect hypersensitivity to flea allergens that is mediated mainly by cellular immunity rather than by IgE.¹⁴ However, this was not the case. One dog had no delayed reaction but a positive immediate reaction with Greer extract. The other had positive immediate and delayed reactions with Greer extract, flea saliva and Cte f 1. It is also possible that levels of IgE in the skin and in the serum are quite different as the Fc epsilon receptor on mast cells binds IgE with a very high affinity, thus possibly leading to a positive reaction with IDT even when the serum IgE level is very low.^{4,14} Allercept® was positive in eight dogs in group 3, which were pet dogs with fleas and no dermatological signs. These control dogs had a high amount of specific flea saliva IgE in the absence of any allergic clinical signs. One study¹² has found no differences in IgE (and also in IgG) patterns between flea-allergic and normal dogs, but this is in contrast to the results of other studies^{1,10,27} in which serum IgE levels were found to be high in flea-allergic dogs and low or undetectable in normal dogs. Differences in study design might explain these discrepancies. For example, procedures conducted in experimental animals can produce results different to those seen in companion animals. In companion animals, exposure to fleas (permanent or intermittent) can be difficult to control. Heterogeneity of canine IgE, as has been suspected in canine AD³⁰, could be another factor. There may be some types of IgE unable to produce clinical signs and even positive IDT. These might be present even in control dogs.

CONCLUSION

Not all flea extracts used as reagents for IDT are equipotent. Skin testing with pure flea saliva provided the best correlation between clinical approach to FAD diagnosis and IDT results but skin testing with recombinant Cte f 1 recognized only 40% of naturally flea-allergic dogs. In this respect, whole-body flea extracts were less reliable than flea saliva although the Greer extract showed excellent specificity and good sensitivity. In spite of the use of FcεRIα, an extremely specific receptor for IgE, results of the *in vitro* test studied showed many false positive reactions.

The fairly good results obtained with flea saliva IDT in carefully selected groups of dogs suggest that this reagent could also be useful in larger populations (e.g. pruritic dogs in general). This needs further investigation. In spite of its high cost, commercial use of pure flea saliva should perhaps be considered. Its activity seems little affected by dilution and storage duration, provided it is kept at 6 °C.

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Résumé L'objectif de cette étude était d'évaluer l'intérêt des tests *in vivo* et *in vitro* pour le diagnostic de dermatite par allergie aux piqûres de puces, en comparaison de l'anamnèse, de l'examen clinique et de la réponse au traitement insecticide. Des tests intradermiques avec 4 sources différentes d'allergènes de puces et des tests *in vitro* basés sur l'utilisation du RFCErI ont été réalisés sur 15 chiens allergiques aux puces, 15 chiens atopiques et 15 chiens présentant une pulicose sans signe clinique. La sensibilité, la spécificité, les valeurs prédictives positives et négatives des 5 tests ont été calculés et ont montré de grandes disparités. La sensibilité, la spécificité et l'efficacité globale étaient de 27%, 83% et 64% pour l'extrait Isotec, 67%, 90% et 82% pour l'extrait Greer, 93%, 90% et 91% pour la salive de puce, 40%, 90% et 73% pour l'allergène recombinant Cte f1 produit par Heska et 87%, 53% et 64% pour le test de dosage des IgE basé sur le RFCErI. Ces résultats indiquent que les tests cutanés avec un extrait de puces sont plus efficaces pour le diagnostic de DAPP que les tests *in vitro*. En outre, la salive de puce utilisée comme réactif pour IDR a permis d'obtenir les meilleurs résultats en terme de sensibilité, de spécificité et de résultat global, bien que l'extrait de Greer, un extrait de corps totaux, a également permis une bonne corrélation entre les résultats des IDR et l'approche clinique permettant le diagnostic de DAPP.

Resumen El objetivo de este estudio fue evaluar la precisión de las pruebas *in vivo* e *in vitro* en el diagnóstico de la dermatitis alérgica a pulgas en comparación con la historia, síntomas clínicos y respuesta al control de pulgas. Se realizaron pruebas intradérmicas con cuatro fuentes diferentes de alérgeno de pulga y pruebas de IgE basadas en FceRIα en 15 perros alérgicos a pulgas, 15 perros atópicos y 15 perros con infestación de pulgas pero sin mostrar síntomas clínicos sugestivos de enfermedad cutánea. La sensibilidad, especificidad, el valor predictivo negativo, el valor predictivo positivo y la precisión fue calculada para las cinco pruebas y los resultados variaron notablemente. La sensibilidad, especificidad, y precisión general fueron del 27%, 83% y 64% respectivamente para un extracto (Isotec), 67%, 90% y 82% para otro extracto (Greer), 93%, 90% y 91% para la saliva de pulga, 40%, 90% y 73% para el recombinante Cte f 1 ambos producidos por Heska y 87%, 53% y 64% para la prueba de IgE basada en FceRIα. Estos resultados indican que las pruebas intradérmicas con extractos de pulga son más precisas en el diagnóstico de la dermatitis alérgica a las pulgas que las pruebas *in vitro*. Además, la saliva de pulga pura utilizada como reactivo para la prueba intradérmica aportó los mejores resultados en cuanto a sensibilidad, especificidad y precisión general a pesar de que el extracto de Greer y el extracto de pulgas enteras también permitieron una buena correlación entre los resultados de las pruebas intradérmicas y el enfoque clínico al diagnóstico de la dermatitis alérgica a pulgas.

Zusammenfassung Ziel dieser Studie war es, die Genauigkeit von *in vivo* und *in vitro* Tests zur Diagnose von Flohallergiedermatitis im Vergleich zu Vorbericht, klinischen Anzeichen und Erfolg von Flohkontrolle zu überprüfen. Bei 15 flohallergischen Hunden, 15 atopischen Hunden und 15 mit Flöhen befallenen Hunden ohne klinische Symptome einer Hauterkrankung wurden Hauttests mit vier verschiedenen Flohallergenen und FcεRIα-gestützte IgE-Tests durchgeführt. Für alle 5 Tests wurden Sensitivität, Spezifität, negativer Prädiktivwert, positiver Prädiktivwert und Genauigkeit kalkuliert und die Ergebnisse variierten stark. Sensitivität, Spezifität, und Gesamtgenauigkeit waren 27%, 83% beziehungsweise 64% für einen Extrakt (Isotec), 67%, 90% und 82% für einen anderen Extrakt (Greer), 93%, 90% und 91% für Flohspeichel, 40%, 90% und 73% für rekombinantes Cte f 1, beide produziert von Heska und 87%, 53% und 64% für einen FcεRIα-gestützten IgE-Test. Diese Ergebnisse weisen darauf hin, dass IDT mit Flohextrakten für die Diagnose von FAD genauer sind, als *in vitro* Tests. Außerdem lieferte reiner Flohspeichel als Reagenz für den IDT die besseren Ergebnisse, was Sensitivität, Spezifität, und Gesamtgenauigkeit anbelangt, obwohl der Greer-Extrakt, ein Ganzkörper-Flohextrakt, auch eine gute Korrelation zwischen IDT-Ergebnissen und klinischem Zugang zur Diagnose der FAD erlaubte.