

22204

Flea allergy dermatitis in dogs diagnosed by intradermal skin tests

R. STOLPER, 3420 Herzberg Harz, Junkernstrasse 11, Germany, J. P. OPDEBEECK, Department of Parasitology, The University of Queensland, Brisbane 4072, Queensland, Australia

Thirty-three dogs were categorised according to their clinical signs of flea allergy dermatitis and reactivity to feeding fleas (*Ctenocephalides felis felis*). A soluble extract of whole fleas (FS), fractions of this extract separated by chromatography, and a commercially available flea antigen extract were used in intradermal skin tests (IDST) to establish the presence or absence of type I and type IV hypersensitivity. The reactions were measured and the results were analysed using three grading systems commonly reported in the literature. The results of the IDST for the groups of dogs varied according to the grading system used. FS, the most effective of the antigen preparations, identified 94 per cent of dogs which reacted to feeding fleas when a result was considered positive if the mean diameter of the wheal at the antigen injection site exceeded the diameter of the wheal at the negative control site by five mm at 15 and/or 30 minutes after injection.

episode in a hypersensitive host (Baker and Thomsett 1990), this method must be considered to have limited value. Although the intradermal skin test (IDST) is technically the most difficult skin test to perform, it is also the most reliable and reproducible (Chamberlain and Baker 1974).

In preliminary work in the authors' laboratory, semi-purified soluble flea antigens (FS) were used in the IDST with encouraging results (Slacek and Opdebeeck 1993): 67 per cent of the dogs that reacted to challenge with feeding fleas reacted to this soluble flea antigen. The FS have been further purified by size exclusion chromatography and the fractionated and reconstituted antigens, and a commercially available flea extract have been tested in the IDST. The selection of trial animals in this study was changed and their reactivity was scored by means of three grading systems commonly reported in the literature, to assess the effect of these grading systems on the diagnosis.

Materials and methods

Flea antigens

A colony of fleas (*Ctenocephalides felis felis*), free of *Dipylidium caninum* cysticercoids, were maintained on cats and in the laboratory. Adult unfed fleas were harvested and stored at -70°C. FS was prepared as described by Slacek and Opdebeeck (1993). The protein concentration of this flea extract was measured by a modified Lowry assay (Markwell et al 1978). A commercial flea antigen (Greer Laboratories) was also used in the IDST.

The FS was separated by high performance size exclusion chromatography on a Waters System (System controller 721, Wisp 710 B; Millipore) using a Waters Protein-PAK Sw 300 preceded by a guard column. The mobile phase, sterile 0.15 M

HYPERSENSITIVITY to the bites of fleas, defined as flea allergy dermatitis (FAD) (Halliwell 1985), is the most common allergic skin disease of dogs and cats (White and Ihrke 1983, Muller et al 1989, Reedy and Miller 1989). In dogs, the clinical signs of FAD are a pruritic papulocrustous dermatitis with the typical skin lesions occurring on the dorsal lumbosacral area (Nesbitt 1983, Scheidt 1988). Although the diagnosis of FAD is frequently based on the clinical signs (Kieffer and Kristensen 1979), the disease can mimic almost any pruritic dermatosis (Halliwell and Gorman 1989), and a definitive test for FAD is therefore desirable for its correct diagnosis. An evaluation of the response to a complete flea control programme is the most widely used method of diagnosis (Nesbitt 1983), but since a single flea may elicit a severe pruritic

phosphate buffered saline (PBS) pH 7.2, was applied at a flow rate of 1 ml minute⁻¹ and a pressure less than 500 psi. The detector (Waters, Model 440) was set at a wavelength of 280 nm. All the samples were filtered through a 0.45 µm pore filter before being injected on to a column. Molecular weights were estimated from a standard curve made by using molecular weight markers ranging from 12.4 kDa to 669 kDa (Sigma Chemical). The void volume of the column was determined with blue dextran (2000 kDa) (Sigma Chemical). The FS was reconstituted after the chromatography by mixing equal volumes of the separated fractions. This reconstituted preparation was designated FS-recon and tested as an allergen in the IDST. The antigen peaks were cut out and weighed as described by Ullmann and Burtis (1986) to measure their percentage representation within the FS.

Experimental animals

Thirty-six adult dogs of different breeds, ages and of both sexes were obtained from local dog shelters. They were categorised in terms of the presence or absence of clinical signs of FAD (FAD+ or FAD-) which was identified as a pruritic papulocrustous dermatitis confined to the lumbosacral area, caudomedial thighs, ventral abdomen, flanks and neck (Muller et al 1989). The dogs were also categorised according to their reaction to challenge with 15 to 20 live fleas which were allowed to feed for 15 minutes (Slacek and Opdebeeck 1983).

IDST with flea antigen

The IDST were carried out by a method similar to that described by Slacek and Opdebeeck (1993); FS and FS-recon were administered at a dose of 1 µg in 60 µl. The fractions obtained by chromatography were administered at doses which were representative of their percentage weight within the FS chromatogram. Greer antigen was administered at a dose recommended by the manufacturer, ie, 1:1000 final concentration (diluted in PBS).

The 33 dogs tested in the IDST were injected with histamine phosphate (1 mg ml⁻¹) (David Bull Laboratories) diluted 1:100,000 in PBS. In addition, 24 of these dogs received this positive control preparation diluted in 1:10,000 in PBS. All the

dogs were also injected with 60 µl of PBS. A distinct palpable bleb in the skin was used as evidence of a correct injection procedure. None of the dogs were discarded as a result of their reactions to the positive and negative controls.

Assessment of IDST

A positive reaction to feeding fleas (ff+) was identified by multiple, visible and palpable wheals on the dog's skin. A negative reaction to feeding fleas (ff-) was identified by small visible red bite lesions at the sites of the fleas' bites. The reactions to the injected antigens were measured in mm by using a caliper ruler 15 and 30 minutes after the injections. Delayed hypersensitivity reactions were assessed 24 hours after the IDST procedure. The three different grading systems most commonly described in the literature were used to assess the reactions. The first system defined a positive reaction as being at least 3 mm larger than the bleb at the site of injection of PBS (negative control) (Nesbitt and Schmitz 1978, Edmonds 1980, Van Winkle 1981, Nesbitt 1983, Reedy and Miller 1989). The second grading system defined a positive reaction as being at least 5 mm larger than the bleb at the site of the negative control (Baker 1971, Halliwell and Schwartzman 1971, Kieffer and Kristensen 1979, Willemse and Van den Brom 1982, Rosenhagen and Hoffmann 1986, Willemse 1986). The third grading system defined a positive reaction as either equal to or larger than a reaction halfway between the reactions at the sites of injection of the positive and negative controls (Scott 1981, Nesbitt 1983, Muller et al 1989).

Statistical analyses

An analysis of variance and Student's *t* test (Sokal and Rohlf 1987) were used to analyse the significance of the results.

Results

Experimental animals

All the dogs had signs of flea infestation established by the presence of either living fleas or flea faeces. Of the 36 dogs exposed to feeding fleas, 17 had developed wheals within 15 minutes and were classified as ff+; no reaction to challenge (ff-) was observed in 16 of the dogs and three had equivocal

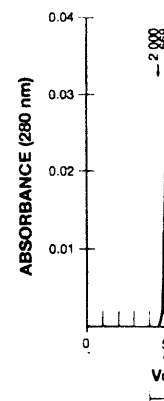


FIG 1: Chromatography of flea antigen. The peak at 4.5 ml is indicated by arrow.

reactions and wheals. There were 10 dogs in each category.

Chromatography

FS was separated into 15 fractions. The fractions were administered in IDST at doses of 1 µg 60 µl⁻¹; fraction D 15 was administered at 1 µg 60 µl⁻¹.

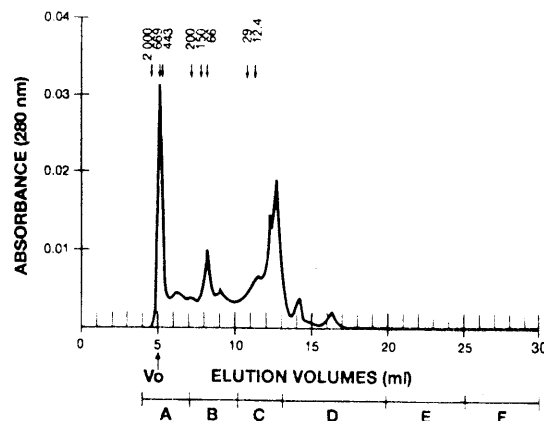


FIG 1: Chromatogram of FS antigens separated by size exclusion chromatography. Fractions designated A-F were used in intradermal skin tests. Vo = void volume. Molecular weights (kDa) are indicated by arrows above the chromatogram.

reactions and were not used in further experiments. There was no statistical difference between the numbers of fleas that fed on the groups of dogs categorised as ff+ and ff-.

Chromatographic separation of FS

FS was separated into three major peaks (Fig 1). The fractions designated A, B, C and D were used in IDST at doses which reflected their percentage representation in FS: fraction A 28.1 per cent, 0.28 µg 60 µl⁻¹; fraction B 24.1 per cent, 0.24 µg 60 µl⁻¹; fraction C 32.6 per cent, 0.33 µg 60 µl⁻¹; fraction D 15.2 per cent, 0.15 µg 60 µl⁻¹.

Fractions E and F were used in the IDST in the form they were collected from the chromatographic column; owing to the low levels of protein present they could not be measured accurately. No proteins were eluted in the void volume and the extract therefore contained proteins of molecular weight ≤2000 kDa. The third major protein peak contained low molecular weight proteins (≤12.4 kDa).

IDST with FS, fractionated FS, FS-recon and commercial flea antigen

Blebs with a range of 5 to 7 mm in diameter were apparent upon the injection of 60 µl PBS in all the dogs. The PBS was completely resorbed at different times in different animals, usually 30 to 60 minutes after the injection. In the 33 dogs tested, histamine phosphate (1:100,000) caused a mean (SEM) skin reaction of 10.6 (0.2) mm (range 8.5 to 13.8 mm) after 15 minutes, and 10.8 (0.2) mm (range 8.5 to 14.3 mm) after 30 minutes. In 24 of the dogs, histamine phosphate diluted 1:10,000 caused reactions of 13.6 (0.3) mm (range 10.5 to 15.5 mm) after 15 minutes and 14.4 (0.4) mm (range 11.3 to 17.3 mm) 30 minutes after the injection.

In the animals with either non- or poorly pigmented skin, the reactions which occurred within 30 minutes of the injection of the flea antigens consisted of erythematous wheals. The results obtained in the group of 33 dogs by using the three grading systems are shown in Tables 1, 2 and 3,

TABLE 1: Numbers of dogs and percentages (in brackets) reactive in an IDST to FS, fractions of FS and a commercial flea extract. A result was considered positive when the mean diameter of the wheal at the site of antigen injection was at least 3 mm larger than the mean diameter of the bleb at the site of injection of PBS*

Antigen	Response of dogs to feeding fleas					
	Positive clinical signs		Total (16)	Negative clinical signs		Total (15)
	Total (17)	FAD+ (9)		FAD+ (1)	FAD- (15)	
Greer	12 (71)	6 (67)	6 (75)	5 (31)	NC	4 (27)
A	—	—	—	3 (9)	NC	3 (20)
B	11 (65)	5 (56)	6 (75)	2 (13)	NC	2 (13)
C	13 (77)	7 (78)	6 (75)	6 (38)	NC	5 (33)
D	5 (29)	4 (44)	1 (13)	—	NC	—
E	6 (35)	4 (44)	2 (25)	—	NC	—
F	5 (29)	4 (44)	1 (13)	—	NC	—
FS-rec	15 (94)	8 (89)	8 (100)	5 (31)	NC	5 (33)
FS	17 (100)	9 (100)	8 (100)	10 (63)	NC	9 (60)

* Nesbitt and Schmitz (1978), Edmonds (1980), Van Winkle (1981), Nesbitt (1983), Reedy and Miller (1989).

Greer Greer Flea Antigen, Greer Laboratories, USA, A-F Fractions from FS separated by size exclusion chromatography, FS-rec FS reconstituted from fractions, NC Not calculated.

of PBS. A dis-
used as evi-
e. None of the
their reactions

as (ff+) was
dipable wheals
on to feeding
visible red bite
The reactions
asured in mm
minutes after
ivity reactions
ST procedure.
is most com-
were used to
em defined a
mm larger than
PBS (negative
78, Edmonds
83, Reedy and
ystem defined
t 5 mm larger
gative control
artzman 1971,
emse and Van
offmann 1986,
ystem defined
or larger than
actions at the
negative con-
ter et al 1989).

udent's *t* test
to analyse the

estation estab-
ing fleas or flea
eding fleas, 17
utes and were
nge (ff-) was
had equivocal

TABLE 2: Numbers of dogs and percentages (in brackets) reactive in an IDST to FS, fractions of FS and a commercial flea extract. A result was considered positive when the mean diameter of the wheal at the site of antigen injection was at least 5 mm larger than the mean diameter of the bleb at the site of injection of PBS*

Antigen	Response of dogs to feeding fleas					
	Positive clinical signs			Negative clinical signs		
	Total (17)	FAD+ (9)	FAD- (8)	Total (16)	FAD+ (1)	FAD- (15)
Greer	7 (41)	2 (22)	5 (63)	2 (13)	NC	2 (13)
A	—	—	—	—	NC	—
B	6 (35)	2 (22)	4 (50)	—	NC	—
C	10 (59)	6 (67)	4 (50)	—	NC	—
D	—	—	—	—	NC	—
E	6 (35)	4 (44)	2 (25)	—	NC	—
F	2 (12)	1 (11)	1 (13)	—	NC	—
FS-rec	13 (77)	6 (67)	7 (88)	1 (6)	NC	1 (7)
FS	16 (94)	8 (89)	8 (100)	—	NC	—

* Baker (1971), Halliwell and Schwartzman (1971), Kieffer and Kristensen (1979), Willemsse and Van den Brom (1982), Rosenhagen and Hoffmann (1986), Willemsse (1986)
Greer Greer Flea Antigen, Greer Laboratories, USA, A-F Fractions from FS separated by size exclusion chromatography, FS-rec FS reconstituted from fractions, NC Not calculated

TABLE 3: Numbers of dogs and percentages (in brackets) reactive in an IDST to FS, fractions of FS and a commercial flea extract. A result was considered positive when the mean diameter of the wheal at the site of antigen injection was equal to or larger than a reaction halfway between the reactions at the sites of injection of the positive (histamine 1:100,000) and negative control (PBS)*

Antigen	Response of dogs to feeding fleas					
	Positive clinical signs			Negative clinical signs		
	Total (17)	FAD+ (9)	FAD- (8)	Total (16)	FAD+ (1)	FAD- (15)
Greer	12 (71)	5 (56)	7 (88)	9 (56)	NC	8 (53)
A	—	—	1 (13)	2 (13)	NC	2 (13)
B	13 (77)	7 (78)	6 (75)	5 (31)	NC	4 (27)
C	15 (88)	8 (89)	7 (88)	6 (38)	NC	5 (33)
D	5 (29)	3 (33)	2 (25)	1 (6)	NC	1 (7)
E	7 (41)	5 (56)	2 (25)	—	NC	—
F	5 (29)	4 (44)	1 (13)	1 (6)	NC	—
FS-rec	17 (100)	9 (100)	8 (100)	10 (63)	NC	10 (67)
FS	17 (100)	9 (100)	8 (100)	11 (69)	NC	10 (67)

* Scott (1981), Nesbitt (1983), Muller et al (1989)
Greer Greer Flea Antigen, Greer Laboratories, USA, A-F Fractions from FS separated by size exclusion chromatography, FS-rec FS reconstituted from fractions, NC Not calculated

and are given as the numbers and percentages of positive responses in both ff groups and in their clinical subgroups. Only one ff- dog had signs of FAD and therefore no calculation was done in this clinical subgroup (ff-, FAD+). Only two dogs (both ff+) exhibited delayed hypersensitivity reactions (red indurated nodules, 2 to 3 mm in diameter) to FS, FS-recon and the commercial antigen. One of these dogs also exhibited delayed hypersensitive reactions to FS fractions B, C and D. Multiple nodules were also visible at the flea feeding sites in both dogs 24 hours after exposure.

Discussion

In a survey of practices used in the IDST, De

Boer (1989) showed that different parameters were used by clinicians to establish and to evaluate the results of an IDST. In this study, the results of the IDST were assessed by the three grading systems most commonly described in the literature. The best results in terms of the proportion of true positive reactions were obtained by using the grading system in which a positive reaction was defined as being at least 5 mm larger than the bleb at the site of the negative control (PBS); almost all the dogs which were hypersensitive to feeding fleas were identified by FS (94 per cent) and no false positives occurred in the ff- group (Table 2). The grading system that defined a positive reaction as being at least 3 mm larger than the bleb at the site of injection of PBS would have classified

100 per cent IDST; however, it also have been used to assess Edmonds 19 Reedy and 2 tive reaction greater than tions at the negative control were correct but 67 per cent was classified as hyper

In prelin described the average diameter of histamine wheal exceeded by the wheal at control solution which did not from the study this criterion only 58 per cent detected wheal (2) was used from evaluation mine reactivity diluted 1:100

Several authors have used FAD to distinguish sensitive dogs. Winkle 198 sent study, e of FAD had reactions to of clinical e judgements. Moreover, the FAD on a clinical because almost FAD (Halliwell false positive

Crude extract was used with nodules which ties (McGib that crude was used in a serial (1990) detected kDa isolated

100 per cent of the ff+ dogs as positive to FS in the IDST; however, 63 per cent of the ff- dogs would also have been falsely designated as flea allergic (Table 1). This grading system has frequently been used to assess IDST (Nesbitt and Schmitz 1978, Edmonds 1980, Van Winkle 1981, Nesbitt 1983, Reedy and Miller 1989). Similarly, when a positive reaction was defined as either equal to or greater than a reaction halfway between the reactions at the sites of injection of the positive and negative controls, 100 per cent of the ff+ dogs were correctly recognised as allergic to FS antigen, but 67 per cent of the ff- dogs were falsely identified as hypersensitive (Table 3).

In preliminary work with FS, the authors described the results obtained in dogs in which the average diameter of the wheal at the site of injection of histamine phosphate (diluted 1:100,000) exceeded by at least 5 mm the average diameter of the wheal at the site of injection of a negative control solution (sterile 0.15 M PBS, pH 7.2). Dogs which did not meet this criterion were discarded from the study (Slacek and Opdebeeck 1993). If this criterion had been applied in the present study only 58 per cent of the ff+ dogs would have been detected when the second grading system (Table 2) was used. Hence dogs should not be excluded from evaluation in an IDST on the basis of histamine reactivity, especially when the histamine is diluted 1:100,000.

Several authors have used clinical evidence of FAD to distinguish between normal and hypersensitive dogs (Kristensen and Kieffer 1978, Van Winkle 1981, Moermans et al 1988). In the present study, eight of 23 dogs with no clinical signs of FAD had significant type 1 hypersensitivity reactions to feeding fleas. Thus using the criterion of clinical evidence of FAD is likely to render the judgements on IDST grading systems invalid. Moreover, the designation of a dog as positive for FAD on a clinical basis alone can be problematic because almost any pruritic dermatitis can mimic FAD (Halliwell and Gorman 1989) and result in a false positive diagnosis.

Crude extracts of parasites may be contaminated with non-specific immunomodulatory molecules which may obscure their allergenic properties (McGibbon et al 1990). It seems advisable that crude whole body flea extracts should not be used in a sensitive test like an IDST. McGibbon et al (1990) determined that a protein molecule of 25 kDa isolated by size exclusion chromatography

from a crude *Ascaris suum* body fluid extract was responsible for more than 80 per cent of the allergic properties of the preparation. In the study reported here, none of the fractionated antigens were as reactive in the IDST as FS, regardless of which system was used to grade the reaction. Reconstituted FS made from the pooled fractions also identified fewer ff+ dogs as hypersensitive (compared with FS), suggesting that the allergen(s) had been damaged during their passage through the chromatographic column. None of the separated antigens were deemed satisfactory in detecting hypersensitive animals; fraction C which identified 59 per cent of ff+ dogs yielded the best results. It appears that most of the allergenic molecules responsible for the positive IDST reaction to FS in flea allergic dogs were present in this fraction (less than 30 kDa). An increased dose of fraction C would be unlikely to have yielded better results because there was a high reactivity in ff- dogs to this preparation when the two other grading systems were used, ie, an increase in dose is likely to have resulted in an increase in false positive results.

Histamine phosphate diluted 1:100,000 was used in all the dogs tested by the IDST and after being diluted at 1:10,000 in 24 of them. Many of the ff- dogs would have been wrongly diagnosed as allergic to flea antigens, if a grading system using histamine at 1:100,000 as a positive control had been used to interpret the IDST results (Table 3). The number of false positive dogs would have been even greater if a histamine concentration of 1:10,000 had been used to determine the cut-off point (data not shown). Histamine diluted to various concentrations has frequently been used as a positive control in the IDST. A dilution of 1:100,000 was used by Reedy (1982), Nesbitt et al (1984), Scheidt (1988) and Muller et al (1989). Other authors used a dilution of 1:10,000 of histamine phosphate (Rosenhagen and Hoffmann 1986, Willemse 1986, Moermans et al 1988). A study in which the IDST was evaluated in dogs revealed that dilutions of histamine at 1:100,000 did not yield reproducible reactions (Willemse and Van den Brom 1982).

In this study, false positive reactions occurred in response to some of the antigens tested, regardless of which of the three systems was used to grade the responses. There are several reasons for false positive reactions in the IDST. Irritants in whole flea extracts seems to be a major problem (Reedy

parameters
and to evalu-
y, the results
grading sys-
the literature.
rtion of true
using the
reaction was
than the bleb
; almost all
to feeding
ent) and no
p (Table 2).
positive reac-
the bleb at
e classified

1982) and Slavin (1974) indicated that irritants with low molecular weights in allergen preparations can cause false positive reactions in the IDST. Although 63 per cent of the ff- dogs would have been designated as hypersensitive (false positives) if the grading system using a 3 mm or greater difference between test and control sites had been used for FS, it is notable that no false positives were recorded with FS when the grading system used a difference of 5 mm or more. This confirms that a rigorous cut-off system should be applied to establish a positive response in the IDST. Most practitioners evaluate the IDST by using subjective grading methods (Nesbitt et al 1984), for example, by the observation and palpation of skin reactions. In view of the results reported here, both the commercial Greer antigen and FS would result in some false positive interpretations of skin tests under these circumstances; thus it is strongly recommended that research workers and practitioners should measure skin responses to allergens by using a caliper ruler and a differential of at least 5 mm between the test and the control injection as evidence of a positive response.

A positive IDST to allergens is proof of the presence of skin-sensitising antibodies, but does not necessarily mean that these antibodies are relevant to the pathogenesis of the disease (De Boer 1989). Positive reactions to the IDST in human beings can persist for a long time after clinical symptoms have disappeared and may also be present years before the signs of a clinical allergy develop (Scott 1981). This could explain the positive IDST and flea feeding tests in the eight dogs that did not exhibit clinical signs of FAD. Up to 50 per cent of the human population react to one or more atopic allergens (Scott 1981), and in areas where fleas are endemic, 40 per cent of clinically normal dogs and cats with no signs of FAD have positive IDST results to flea antigen (Muller et al 1989). It appears either that false positive reactions occur or that positive IDST results in clinically normal animals indicate that hypersensitivity is developing. Further studies are needed to investigate the antigen(s) in flea extracts such as FS which are responsible for allergy and for their activity in the IDST and disease, and to help to elucidate the pathogenesis of FAD.

Acknowledgements

The authors are grateful to Paravax, Fort

Collins, Colorado, for their generous support of this work. Thanks are due to Professors C. Dobson and M. Rommel who helped make these studies possible.

References

- BAKER, K. P. (1971) Intradermal tests as an aid to the diagnosis of skin disease in dogs. *Journal of Small Animal Practice* **12**, 445-452
- BAKER, K. P. & THOMSETT, L. R. (1990) Canine and Feline Dermatology. Oxford, Blackwell Scientific, pp 161-165
- CHAMBERLAIN, K. W. & BAKER, E. (1974) Diagnostic methods in allergic disease. *Veterinary Clinics of North America* **4**, 47-56
- DE BOER, D. J. (1989) Survey of intradermal skin testing practices in North America. *Journal of the American Veterinary Medical Association* **195**, 1357-1363
- EDMONDS, M. J. (1980) Preliminary investigations into the use of allergy skin testing solutions and desensitising vaccines in dogs. *Journal of Small Animal Practice* **21**, 153-167
- HALLIWELL, R. E. W. (1985) Flea allergy: Pathogenesis, therapy, and flea control. *Proceedings of the American Animal Hospital Association* **52**, 145-149
- HALLIWELL, R. E. W. & GORMAN, N. T. (1989) Veterinary Clinical Immunology. Philadelphia, W. B. Saunders, pp 253-284
- HALLIWELL, R. E. W. & SCHWARTZMAN, R. M. (1971) Atopic disease in the dog. *Veterinary Record* **89**, 209-214
- KIEFFER, M. & KRISTENSEN, S. (1979) Flea hypersensitivity in dogs and cats. *International Journal of Dermatology* **18**, 707-712
- KRISTENSEN, S. & KIEFFER, M. (1978) A study of skin diseases in dogs and cats V. The intradermal test in the diagnosis of flea allergy in dogs and cats. *Nordisk Veterinary Medicine* **30**, 414-423
- McGIBBON, A. M., CHRISTIE, J. F., KENNEDY, M. W. & LEE, T. D. G. (1990) Identification of the major *Ascaris* allergen and its purification to homogeneity by high-performance liquid chromatography. *Molecular and Biochemical Parasitology* **39**, 163-172
- MARKWELL, M. A. K., HAAS, S. M., BIEBER, L. L. & TOLBERT, N. E. (1978) A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Annals of Biochemistry* **87**, 206-210
- MOERMANS, J., MATTHEEWS, D. & VERCRUYSE, J. (1988) Flea-allergy dermatitis in the dog. *Animals Familiars* **3**, 15-21
- MULLER, G. H., KIRK, R. W. & SCOTT, D. W. (1989) Small Animal Dermatology, 4th edn. Philadelphia, W. B. Saunders, pp 166-168, 416-418
- NESBITT, G. H. (1983) Canine and Feline Dermatology. Philadelphia, Lea & Febiger, pp 47-67
- NESBITT, G. H., KEDAN, G. S. & CACIOLO, P. (1984) Canine atopy Part I. Etiology and diagnosis. *Compendium on Continuing Education for the Practising Veterinarian* **6**, 73-84
- NESBITT, G. H. & SCHMITZ, J. A. (1978) Fleabite allergic dermatitis: A review and survey of 330 cases. *Journal of the American Animal Hospital Association* **173**, 282-288
- REEDY, L. M. (1982) Results of allergy testing and hyposensitisation in selected feline skin diseases. *Journal of the American Animal Hospital Association* **18**, 618-623
- REEDY, L. M. & MILLER, W. H. (1989) Allergic Skin Diseases of Dogs and Cats. Philadelphia, W. B. Saunders, pp 81-109, 171-187
- ROSENHAGEN, D. & HOFFMANN, G. (1986) Zum Intrakutantest beim Hund - ein Überblick über 112 Fälle. *Kleintierpraxis* **31**, 131-136
- SCHEIDT, V. J. (1988) Flea allergy dermatitis. *Veterinary Clinics of North America: Small Animal Practice* **18**, 1023-1042
- SCOTT, D. W. (1981) Observations on canine atopy. *Journal of the American Animal Hospital Association* **17**, 91-100
- SLACEK, B. & OPDEBEECK, J. P. (1993) Reactivity of dogs and cats to feeding fleas and to flea antigens injected intradermally. *Australian Veterinary Journal* **70**, 313-314
- SLAVIN, R. G. (1974) Skin tests in the diagnosis of allergies of the immediate type. *Medical Clinics of North America* **58**, 65-69

SOKAL, R. R.
edn. New Y.
ULLMANN, I.
chromatogr.
Philadelphia
VAN WINKL.
intradermal
American A
WHITE, S. D

- SOKAL, R. R. & ROHLF, F. J. (1987) Introduction to Biostatistics, 2nd edn. New York, Freeman and Company, pp 171-173.
- ULLMANN, D. & BURTIS, C. A. (1986) High-performance liquid chromatography. In Textbook of Clinical Chemistry, Ed N. W. Tietz, Philadelphia, W. B. Saunders, pp 159-172.
- VAN WINKLE, K. A. (1981) An evaluation of flea antigens used in intradermal skin testing for flea allergy in the canine. *Journal of the American Animal Hospital Association* **17**, 343-354.
- WHITE, S. D. & IHRKE, P. J. (1983) Allergic Diseases. In Feline Medicine, Ed P. W. Pratt, Santa Barbara, American Veterinary Publications, pp 577-580.
- WILLEMSE, T. (1986) Atopic skin disease: a review and a reconsideration of diagnostic criteria. *Journal of Small Animal Practice* **27**, 771-778.
- WILLEMSE, T. & VAN DEN BROM, W. E. (1982) Evaluation of the intradermal allergy test in normal dogs. *Research in Veterinary Science* **32**, 57-61.

Received June 8, 1993
Accepted November 30, 1993

the support of
Dr C. Dobson
for these studies

the diagnosis of skin
12, 445-452

Canine and Feline
1-165

gnostic methods in
12 **4**, 47-56

testing practices in
Veterinary Medical

into the use of
vaccines in dogs.

osis, therapy, and
Animal Hospital

Veterinary Clinical
12, 284

M. (1971) Atopic

sensitivity in dogs
707-712

of skin diseases
diagnosis of flea
Medicine **30**, 414-

M. W. & LEE.
allergen and its
liquid chromato-
9, 163-172

L. & TOLBERT.
re to simplify pro-
samples. *Annals of*

LYSSE, J. (1988)
is **3**, 15-21

89) Small Animal
lers, pp 166-168,

gy, Philadelphia,

84) Canine atopy
on Continuing

lergic dermatitis:
American Animal

posensitisation in
American Animal

Skin Diseases of
109, 171-187

trakutantest beim
31, 131-136

inary Clinics of
2

Journal of the

of dogs and cats
nally. *Australian*

allergies of the
65-69