

Prevalence of house dust mites and dermatophagoides group 1 antigens collected from bedding, skin and hair coat of dogs in south-west England

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Abstract The house dust mites *Dermatophagoides farinae* (Df) and *D. pteronyssinus* (Dpt) are commonly implicated as allergens causing canine atopic dermatitis in the UK. However, there are few studies that characterize the exposure of UK pet dogs to these mites. The objectives of this study were to determine the prevalence of the mite species on the skin, hair coat and bedding of a population of pet dogs. Dust samples ($n = 68$) were collected from both dogs and their beds using a standardized vacuuming technique and stored at -20°C . Mites were identified using accepted morphological criteria. House dust mite allergen concentrations were assayed using standardized ELISA for Dpt and Df group 1 allergens (Der p 1 and Der f 1). Mites were identified in 15/68 samples (22%) and Dpt was the most common. Df mites were not present. Der p 1 allergens were detected in 60% of samples, and Der f 1 in 6% of samples. There were no significant differences between the number of Der p 1 positive samples from dogs and the number of those from their bedding, or between the average Der p 1 concentrations from dogs and the number of those from their bedding. Contrary to studies elsewhere in Europe and the USA, these findings support studies of human asthma patients in the UK, where exposure to Df is rare, but to Dpt is common. As the prevalence of positive intradermal and serological reactions to Df in atopic dogs is high, further investigations are warranted to clarify true Df hypersensitivity or potential immunological cross-reactivity between mite allergens.

INTRODUCTION

The role of mites in house dust allergies was first suggested by Dekker in 1928. Dekker found large numbers of mites in mattress dust and recorded that human patients with asthma symptoms improved when placed in a mite-free environment.¹ Allergies to house dust mites are a common cause of allergic skin disease in both humans and dogs, and sensitivity to *Dermatophagoides pteronyssinus* (*D. pteronyssinus*) is widely accepted as being the most common cause of asthma and atopic eczema in humans.² Hypersensitivity to *Dermatophagoides* spp. is also considered to be a major factor in the pathogenesis of canine atopic dermatitis.³

The pyroglyphid mite *D. pteronyssinus* predominates over most house dust mite (HDM) species worldwide. Some mites are unique to certain countries, and the distribution of others, such as *D. farinae* and *Euroglyphus maynei*, vary according to climatic conditions. *D. farinae* is most common in dry, continental climates, is rare in coastal climates such as the British Isles,

absent from Central Africa and Cuba, and is not fully established in terms of prevalence in some geographical areas.⁴ Studies in areas where *D. farinae* is ubiquitous report a peak in mite populations in August. In comparison, *D. pteronyssinus* populations in the same areas peak in July, and then decrease in October.⁵ Optimal development of *D. farinae* populations occurs at temperatures of $25\text{--}30^{\circ}\text{C}$ and 50–60% relative humidity and the life cycle is approximately 30 days in duration.⁶

D. pteronyssinus, *D. farinae* and, more recently, storage mites^{3,7–11} have been investigated as antigens in the pathogenesis of canine atopic dermatitis. Data from intradermal and serological testing suggest that *D. farinae* is the most common antigen implicated in canine atopic dermatitis in the UK,^{3,12–14} Europe,^{11,15,16} the USA,^{17,18} Australia¹⁹ and Japan.²⁰ However, studies investigating the prevalence of HDM species in UK homes rarely identify *D. farinae*, if at all.^{21–26} As exposure is necessary to achieve sensitization, it follows that dogs are exposed to a different mite spectrum than humans in their microenvironment, or that *D. farinae* is more antigenic to the canine immune system than the human immune system and so minimal amounts of the antigen trigger allergic reactions in dogs, or that cross-reaction with other allergens results in positive reactions to *D. farinae*.

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The objectives of this study were to examine the immediate microenvironment (hair, surface skin debris and bedding) of a selected population of UK dogs to (1) determine the prevalence of *D. farinae* and *D. pteronyssinus* mites; (2) identify house dust and storage mite species using morphological characteristics; and (3) assay the *D. pteronyssinus* and *D. farinae* group 1 allergens (Der p 1 and Der f 1) using standardized ELISA methods.

MATERIALS AND METHODS

Selection of dogs

A convenience population of dogs was recruited from the University of Bristol Small Animal Hospital population and local homes. The major inclusion criteria were the availability of removable bedding for each dog and that neither dog nor bedding were washed for 2 weeks prior to examination. A simple questionnaire was used to collect information on the dog's environment including the amount of time spent indoors, amount of time spent in contact with bedding, coat length (short, long), and on-animal or environmental insecticide/acaricide use.

Collection of dust samples

Dust samples were collected by the same investigator (AJ) between June and October 2003 to coincide with the previously published peaks in house dust mite populations.^{5,22,26} Duplicate samples were obtained from each animal's coat and bedding at the same time. One sample was used for direct microscopy and one for the ELISA. A specialized vacuum cleaner (Medivac Combo, Medivac Healthcare Ltd, London, UK) approved by the British Allergy Foundation was used with a removable dust filter (Mitest Dust Collector, Indoor Biotechnologies, Cardiff, UK). For each dog, the filter was continuously moved over the entire trunk for a 2-min period, and for each bed the bedding was similarly sampled. A new Mitest collector was used for each animal or bed, and the inner filter containing dust and hair was removed and sealed in a plastic bag for storage. Filters were stored as collected at -20°C for between 1 and 6 months until processing.²⁷

Morphological identification of mite species

Filter samples were thawed and 100 mg of fine dust was collected for mite analysis. Whole mites were extracted from the dust samples using a described flotation technique,²⁸ removed from suspension with a mounted needle or fine wire loop and placed on glass slides in Hoyer's medium.²⁹ Prepared slides were kept warm for 24–48 h. Mites were counted and identified using standard morphological criteria.^{2,30,31} Parts of mites, larvae and very desiccated mites were recorded as unclassified. After identification, mites were placed in one of three categories; house dust mites (*D. pteronyssinus*), storage mites (*Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Acarus siro*), or unclassified

(not a house dust or storage mite). Unclassified mites included mite larvae that could not be definitively identified because adult morphological characteristics are used to distinguish the species.

Analysis for Der f 1 and Der p 1 allergens by enzyme-linked immunosorbent assay (ELISA)

Dust samples (50–100 mg) were added to 1–2 mL of 0.05% Tween 20 at pH 7.4, gently agitated for 4 h, centrifuged, and the supernatant collected to make a stock solution of 50 mg mL^{-1} . Samples were stored at -80°C for up to 10 days before processing. Analysis for the house dust mite allergens Der f 1 and Der p 1 was performed using a validated³² commercial monoclonal ELISA according to the manufacturer's protocol (Der f 1 ELISA kit (6A8/4C1)/Der p 1 ELISA kit (5H8/4C1), Indoor Biotechnologies, Cardiff, UK). Allergen concentrations were reported as ng mL^{-1} of the 1 : 10 diluted dust sample stock solution (5 mg mL^{-1}) after interpolation from standard reference curves for each assay, which was made according to the manufacturer's protocol (see above). The allergen density could then be calculated as micrograms gram^{-1} of dust. Der f 1 and Der p 1 ELISAs were defined as positive if the concentration of the sample was above 2 ng mL^{-1} at the 1 : 10 dilution.

Statistical analysis

Statistical analysis was carried out using SPSS ver. 12 (SPSS Inc, Chicago, IL, USA) running on Windows XP Professional. Descriptive statistics and the chi-squared test were used to examine the associations between dog and bedding samples positive for different mite species within the mite microscopy data. For the allergen analysis data, comparisons between different allergens from both dog and bedding samples, and comparisons between dog and bedding samples with the same allergen were made with the chi-squared test (exact statistics) for weighted data. The relationship between allergen concentrations from samples that had been exposed to ectoparasitocides and those that had not been exposed was also examined. The Mann-Whitney test (exact statistics) was used for two independent samples. For all data, *P*-values were two sided and $P < 0.05$ was considered significant.

RESULTS

Selection of dogs

Thirty-four dogs and their bedding were included in the study. Hair type was classified subjectively as long in 12 dogs (Cavalier King Charles spaniel, long haired German shepherd dog, golden retriever, Gordon setter and springer spaniel, Border collie), and short in 22 dogs (boxer, dachshund, Dalmatian, smooth-coated Jack Russell terrier, smooth-coated lurcher, clipped schnauzer, Rhodesian ridgeback, Labrador retriever, Rottweiler and West Highland white terrier). No statistically significant relationship was observed between

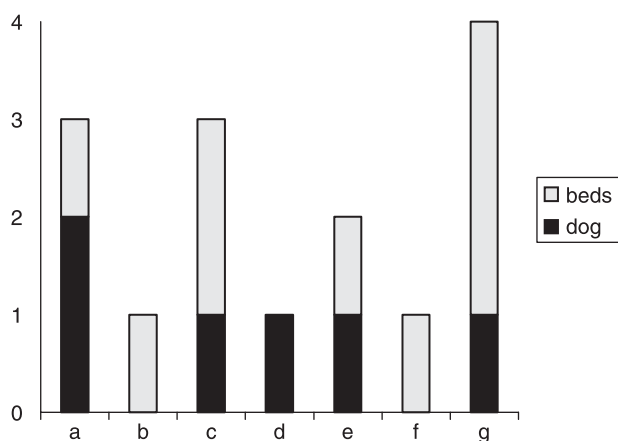


Figure 1. Number of dog and bedding dust samples (y axis) that were positive for different mite species (x axis); a: *D. pteronyssinus* alone; b: *D. pteronyssinus* and storage mite species; c: *D. pteronyssinus* and unclassified mites; d: *D. pteronyssinus*, storage and unclassified mites; e: storage mite species alone; f: storage and unclassified mite species; g: unclassified mites alone.

coat length and mites found or mite allergen concentration during analysis of the data. All dogs spent at least 10 h per day in contact with their bedding.

Analysis of mite species using microscopy

The number of different mite species in dog and dog bedding samples that were identified using direct microscopy are detailed in Fig. 1. Overall, mites were identified in 15/68 (22%) of samples. The difference in number of positive samples between dog and bedding samples was not significant ($P = 0.068$). *D. pteronyssinus* was the single most common mite species found and was identified in 11.8% of samples, either alone or in combination with storage or unclassified mites (Fig. 1). No *D. farinae* mites were identified in any sample. In positive samples, HDM density varied from one to 15 mites per 100 mg ($100\text{--}1500\text{ g}^{-1}$) dust sample. All samples that were positive for *D. pteronyssinus* mites were also positive for Der p 1 allergen.

Storage mite species were identified in 7.4% of samples and multiple unclassified mite species in 13.2% of samples.

Analysis of Der f 1 and Der p 1 allergens

Out of 68 samples, four (5.9%) were positive for Der f 1 (dogs = 2, beds = 2) and 41 (60.3%) samples were positive for Der p 1 (dogs = 22, beds = 19). One sample was positive for Der f 1 alone and 26 samples (38.2%) were negative. The difference between the number of Der p 1 and Der f 1 in positive dog samples was highly significant ($P < 0.000$). The difference between the number of positive Der p 1 and Der f 1 in bedding samples was highly significant ($P < 0.0001$). The differences between dog and bedding samples positive for Der p 1 and dog and bedding samples positive for Der f 1 were not significant ($P = 1.0$ and 0.808 , respectively).

Positive samples ranged from concentrations of 2 ng mL^{-1} ($40\text{ }\mu\text{g g}^{-1}$) to 348 ng mL^{-1} ($6968\text{ }\mu\text{g g}^{-1}$).

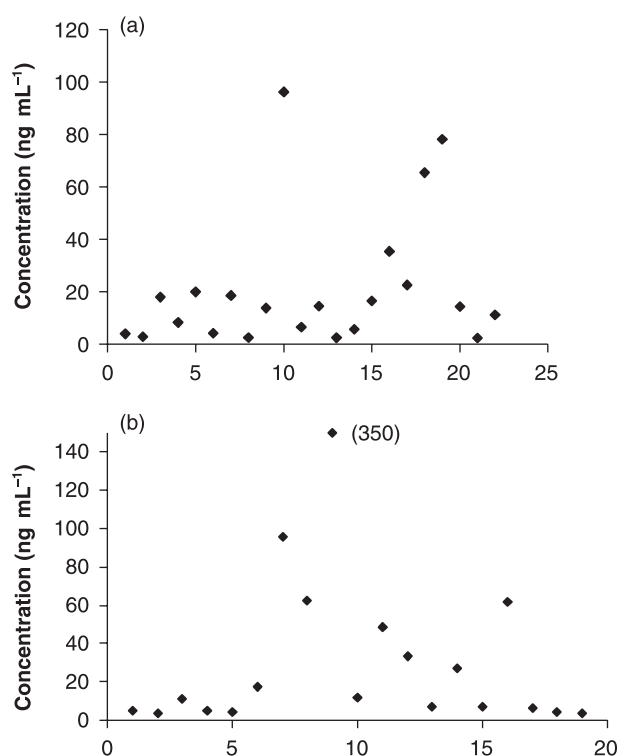


Figure 2. (a) Range of Der p 1 allergen concentrations obtained from individual positive dog dust samples ($n = 22$). (b) Range of Der p 1 allergen concentrations obtained from individual positive bedding dust samples ($n = 19$).

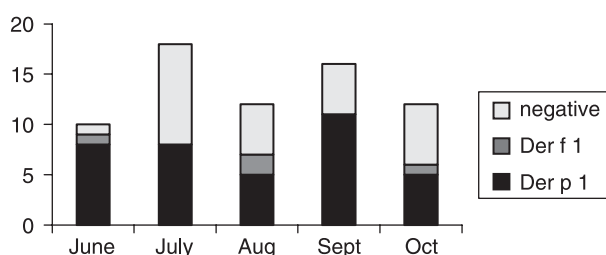


Figure 3. Number of samples (y axis) that were positive for Der p 1 allergen, Der f 1 allergen, or negative (June–October 2003).

Figure 2 (a,b) show the range of allergen concentrations for positive dog and bedding samples. The four Der f 1 positive samples ranged between 2.3 and 11.2 ng mL^{-1} ($46\text{--}224\text{ }\mu\text{g g}^{-1}$).

Effect of month of the year on allergen concentration

Collection of samples was spread fairly evenly throughout the months of June to October 2003. The number of positive and negative allergen samples is illustrated in Fig. 3. There was no statistical significance associated with month of collection, number of samples collected per month, or sample positivity.

Correlation between use of topical ectoparasiticide on dogs and house dust mite allergen concentration
Ectoparasiticide use was reported in 27/34 dogs within the 3-month period prior to sampling. In all cases, topical 'spot-on' prescription only medicines were used

according to manufacturers' instructions. As so few samples were positive for Der f 1, it was not possible to perform any statistical analysis on that data. The mean allergen concentrations of Der p 1 samples in dog and bedding samples with ectoparasiticide were 11 ng mL^{-1} and 9.2 ng mL^{-1} , respectively, and without ectoparasiticide were 21.1 ng mL^{-1} and 72.4 ng mL^{-1} , respectively. The difference between mean allergen concentration of dog and bedding samples was not statistically significant.

DISCUSSION

In this study, *D. pteronyssinus* was the only HDM species found by microscopic examination of dog and bedding dust samples. The number of Der p 1 positive dog and bedding samples was similar, as was the number of Der f 1 positive dog and bedding samples. However, there were a high number of Der p 1 positive samples and very few Der f 1 positive samples. The results of this study show that dogs seem to be exposed to the same spectrum of HDM species as humans are in this region of the UK.

The low prevalence of *D. farinae* mites and Der f 1 allergen in this study compared with *D. pteronyssinus* and Der p 1 is comparable to UK^{21–26} and Australian house dust studies.^{33–35} In contrast, studies of mite fauna in houses in other areas of Europe and the USA commonly find *D. farinae*.^{5,36} In the USA and continental Europe, *D. farinae* is also reported frequently in dogs' microenvironment.^{37–39} In one German study, the presence of a dog in the house was associated with a 2–3-fold increase in house dust Der f 1 allergen levels,⁴⁰ but again no *D. farinae* mites were found in houses where dogs bedding was sampled in the UK.^{24,41} Seasonal variation may have a dramatic effect on house dust mite populations^{5,6,26} and collection of samples in this study was timed to coincide with times during which *D. pteronyssinus* and *D. farinae* populations should have been at their peak if present. The apparent discrepancy of the findings in this study and other UK house dust studies with those elsewhere in Europe may be a result of a combination of climatic and other environmental factors, making the growth of *D. farinae* mite populations unfavourable.

The effect of dog coat length on mite populations or mite allergen levels has not been previously investigated, but there was no apparent effect in this study. The constant temperature and relative humidity of the canine skin and coat (30–60%)^{42,43} should be more favourable for mite populations than the recorded meteorological environmental relative humidity during the months in which samples were taken (61–89%).⁴⁴ Therefore, the coat environment could have been expected to support the maintenance of HDM populations if they were present in the macroenvironment.

The use of ectoparasiticide products with acaricidal activity on dogs in this study was not significantly associated with lower Der p 1 allergen levels, either in dog

or in bedding samples. However, mean allergen concentrations did appear to be much higher in untreated dog and bedding samples and larger sample numbers may reveal significant differences. Environmental acaricides such as benzoyl benzoate have been shown to both reduce house dust mite levels and improve clinical signs in atopic dogs⁴⁵, and both the insecticidal/acaricidal topical treatments used on dogs in this study have activity against other mite species.^{46,47} Although no dogs or bedding samples in the study had specific environmental acaricides applied, the on-animal adulticide and larvicidal products may have had an effect on house dust in the bedding through direct contact from treated hair and skin debris. Further studies investigating the effect of topical on-animal acaricides on house dust mites need to be performed on larger numbers of dogs.

The high proportion of positive intradermal and serological reactions to *D. farinae* in canine atopic patients from geographical areas where prevalence and exposure is very low – based on studies of house dust – is a consistent finding. When compared to geographical areas where *D. farinae* and *D. farinae* hypersensitivity in dogs are common, these findings are difficult to explain. It is possible that dogs have increased sensitivity to *D. farinae* compared to other HDM species,¹⁴ and hence a very low prevalence would result in a high number of allergic individuals. One study suggested that *D. pteronyssinus* is less allergenic to dogs than other HDM species (*D. farinae* and *E. maynei*).⁴⁶ There is certainly evidence that atopic dogs with positive intradermal test reactions to *D. farinae* have increased peripheral blood mononuclear cell responses to *D. farinae* extract compared to normal dogs.⁴⁷ The apparently high prevalence of hypersensitivity to *D. farinae* in atopic dogs could also be explained by cross-reactivity with other allergens. One UK study on atopic children suggested that patients who are sensitive to HDM allergens may produce IgE; the IgE binds to the allergens of mite species that are not in their environment.²¹ Previous studies of human patients, where a combination of serological, molecular and intradermal techniques have been used, include clear demonstrations of cross-reactivity within *Dermatophagoides* species, between *Dermatophagoides* species and other HDM, e.g. *E. maynei*, between HDM and storage mites, between HDM and other mite species and between HDM and other nonmite allergens.^{48–53} In atopic dogs, cross-reactivity has been demonstrated between *D. pteronyssinus* and *D. farinae* allergens in serum.⁵⁴ Other authors did not find evidence for clinically significant cross-reactivity of intradermal test reactions,⁵⁵ although *D. farinae* and *D. pteronyssinus* extracts were the only mite species evaluated. Any of these possibilities may result in multiple nonspecific intradermal and serological test reactions to what are essentially crude *D. farinae* allergen extracts that have yet to be standardized for veterinary use. Further investigation of allergens causing canine atopic dermatitis in the UK should not be extrapolated from findings

in other geographical areas and HDM species other than *D. pteronyssinus* and *D. farinae* should be considered, as well as storage mite species, as potential allergens.

In conclusion, this pilot study has shown that, in the south-west of England, *D. farinae* does not appear to be a common house dust mite and that, as with humans, dogs have greater exposure to *D. pteronyssinus* than any other HDM species in this area. Allergen levels of Der f 1 are also very low when compared with Der p 1. In view of the fact that many atopic dogs have positive intradermal and serological test results to *D. farinae*, further work is needed to clarify whether this reflects true hypersensitivity to *D. farinae* or whether cross-reactivity to other allergens is occurring. Furthermore, if the majority of positive reactions to *D. farinae* in atopic dogs do represent cross-reactivity, these could represent cross-reactions with single or multiple allergens, as is the case in humans. Increased accuracy in identifying allergens to which atopic dogs are responding may result in more successful allergen-specific immunotherapy and allergen avoidance.

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Résumé Les acariens des poussières, *Dermatophagoides farinae* (Df) et *D. pteronyssinus* (Dpt), sont des allergènes fréquemment impliqués dans la dermatite atopique canine au Royaume Uni. Cependant, peu d'études ont caractérisé l'exposition des chiens du RU à ces acariens. Les objectifs de cette étude étaient de déterminer la prévalence des acariens sur la peau, les poils et dans le couchage de chiens. Des prélèvements de poussière (n=68) ont été récoltés de chiens et de leur couchage en utilisant une technique standardisée

d'aspiration, et stockés à -20°C. Les acariens ont été identifiés en utilisant des critères morphologiques. Les taux allergéniques ont été déterminés en utilisant un test ELISA standardisé pour les allergènes du groupe 1 de Dpt et Df (Der p 1 et Der f 1). Des acariens ont été identifiés dans 15/68 prélèvements (22%) et Dpt était le plus fréquent. Df n'a pas été retrouvé. Des allergènes Der p 1 ont été détectés dans 60% des prélèvements et Der f 1 dans 6%. Aucune différence significative n'a été observée entre le nombre de prélèvements positifs pour Der p 1 ni la moyenne des concentrations de Der p 1 chez les chiens en comparaison des prélèvements des lieux de couchage. À l'opposé des données établies ailleurs en Europe ou en Amérique du Nord, ces données confortent les études réalisées chez les patients souffrant d'asthme au RU, pour lesquels l'exposition à Df est rare et celle à Dpt est fréquente. Du fait de la prévalence élevée des tests intradermiques et sérologiques vis à vis de Df chez les chiens atopiques, des études supplémentaires sont nécessaires pour déterminer s'il existe de réelles réactions d'hypersensibilité à Df chez les chiens atopiques, ou s'il s'agit de réactions croisées entre les allergènes d'acariens.

Resumen Los ácaros del polvo doméstico, *Dermatophagoides farinae* (Df) y *D. pteronyssinus* (Dpt), se encuentran frecuentemente implicados como alérgenos que causan dermatitis atópica canina en el Reino Unido (RU). Sin embargo, son pocos los estudios que caractericen la exposición de los perros domésticos del RU a estos ácaros. Los objetivos de este estudio fueron determinar la prevalencia de especies de ácaros en la piel, pelo y cama de una población de perros domésticos. Se recogieron muestras de polvo (n=68) tanto de perros y camas utilizando una técnica estándar de aspiración y se almacenaron a -20°C. Los ácaros fueron identificados utilizando criterios morfológicos aceptados. Se estudiaron las concentraciones de alérgeno de ácaro del polvo doméstico utilizando un ELISA estándar para el grupo 1 de alérgenos de Dpt y Df (Der p 1 y Der f 1). Los ácaros fueron identificados en 15/68 muestras (22%) y Dpt fue el más frecuente. No se identificaron ácaros Df. Se detectaron alérgenos Der p 1 en el 60% de las muestras y Der f 1 en el 6%. No existían diferencias significativas entre el número de muestras positivas de Der p 1 ni en la media de concentraciones de Der p 1 de muestras de perros comparado con las muestras de sus camas. Contrariamente a lo observado en estudios realizados en otras partes de Europa y Norte América, estos hallazgos apoyan los estudios de asma humano en el RU, donde la exposición a Df es infrecuente mientras que es común a Dpt. Teniendo en cuenta la elevada prevalencia de reacciones intradérmicas y serológicas a Df en perros atópicos, serán necesarias nuevas investigaciones para clarificar la existencia de verdadera hipersensibilidad a Df o la existencia de posible reactividad inmunológica cruzada entre alérgenos de ácaros.

Zusammenfassung Die Hausstaubmilben *Dermatophagoides farinae* (Df) und *D. pteronyssinus* (Dpt) sind häufige auslösende Allergene bei caniner atopischer Dermatitis in Großbritannien. Jedoch gibt es nur wenige Studien, die die Exposition mit diesen Milben bei Hunde in Großbritannien untersuchen. Ziel dieser Studie war es, die Prävalenz der Milbenarten auf Haut, Haarkleid und Liegestellen bei einer Population von Haushunden zu bestimmen. Staubproben (n=68) wurden sowohl von den Hunden als auch von ihren Liegestellen mittels einer standardisierten Staubsaugtechnik gesammelt und bei -20°C gelagert. Die Milben wurden anhand akzeptierter morphologischer Kriterien identifiziert. Die Konzentrationen an Hausstaubmilbenallergenen wurden mittels standardisierter ELISA für Dpt- und Df-Gruppe-1-Allergene (Der p 1 und Der f 1) untersucht. In 15/68 Proben (22%) wurden Milben identifiziert und Dpt war die häufigste Milbe. Df-Milben waren nicht vorhanden. Der p 1-Allergene wurden in 60% und Der f 1 in 6% der Proben nachgewiesen. Es gab keinen signifikanten Unterschied weder hinsichtlich der Anzahl von Der p 1 positiven Proben noch hinsichtlich der durchschnittlichen Konzentration von Der p 1 bei den Proben von Hunden im Vergleich zu denen von ihren Liegestätten. Im Gegensatz zu Studien anderswo in Europa und in Nordamerika unterstützen diese Ergebnisse Studien bei humanen Asthmapatienten in Großbritannien, in denen Exposition durch Df selten, durch Dpt jedoch häufig ist. In Anbetracht der hohen Prävalenz von positiven intradermalen und serologischen Reaktionen auf Df bei atopischen Hunden sind weitere Untersuchungen nötig, um wirkliche Df-Hypersensitivität oder mögliche immunologische Kreuzreaktion zwischen Milbenallergenen aufzuhellen.