

Comparison between an intradermal skin test and allergen-specific IgE-ELISA for canine atopic dermatitis

N. Tarpataki¹, B. Bigler², P. Vajdovich¹, K. Vörös¹

¹Department and Clinic of Internal Medicine, Faculty of Veterinary Science, Szent István University, Hungary

²Labor Laupeneck/ imovet bg, Bern, Switzerland

Summary

The aim of this study was to compare the results of an intradermal skin test (IDST) with those of an allergen-specific IgE-ELISA in 210 dogs with atopic dermatitis. All the dogs had a clinical diagnosis of atopic dermatitis and underwent an IDST. The sera of all dogs were analysed for allergen-specific IgE by ELISA using the monoclonal antibody D9 against dog IgE. IDST was used as the standard assay. In both methods, the following antigens provided a positive test result: *Dermatophagoides farinae*, *Acarus siro*, *Tyrophagus putrescentiae*, ragweed, mugwort and *Lepidoglyphus destructor*. ELISA had an overall sensitivity of 82.4% and an overall specificity of 93.8%. The overall accuracy of the ELISA was 91.3%. The evaluated monoclonal D9 ELISA was found to be a reliable tool for the diagnosis of those allergens that cause clinical atopy, and can be recommended for use in dogs when immunotherapy is a therapeutic option.

Keywords: atopic dermatitis, intradermal skin test, ELISA, canine IgE

Atopische Dermatitis beim Hund: Vergleich zwischen Intrakutantest und allergenspezifischem IgE

Zusammenfassung

Das Ziel dieser Studie war es, bei 210 Hunden mit atopischer Dermatitis die Ergebnisse des Intrakutantests (IKT) mit denjenigen eines allergenspezifischen IgE-ELISA zu vergleichen.

Bei 210 Hunden mit der klinischen Diagnose einer atopischen Dermatitis wurde ein IKT durchgeführt. Die entsprechenden Seren wurden mittels eines ELISA auf allergenspezifische IgE untersucht, wobei der monoklonale D9 Antikörper gegen canines IgE verwendet wurde. Bei diesem Vergleich wurden die Werte des IKT als Standard genommen. In beiden Tests wurden mit folgenden Allergenen positive Reaktionen erzielt: *Dermatophagoides farinae*, *Acarus siro*, *Tyrophagus putrescentiae*, Ambrosia (Ragweed), Beifuss und *Lepidoglyphus destructor*. Der ELISA hatte insgesamt eine Sensitivität von 82.4% und eine Spezifität von 93.8%. Die Genauigkeit für alle positiven und negativen Proben betrug 91.3%. Der Gebrauch des monoklonalen Antikörpers D9 erwies sich im ELISA als zuverlässiger Detektionsantikörper zur Messung von allergenspezifischem Hunde-IgE gegen die Allergene, die eine atopische Dermatitis auslösen können. Die Bestimmung der krankheitsauslösenden Allergene ist vor allem dann hilfreich, wenn eine Desensibilisierung angestrebt wird.

Schlüsselwörter: atopische Dermatitis, Intrakutantest, ELISA, Hunde-IgE

Introduction

Atopic dermatitis (AD) is an allergic skin disease of dogs caused by immunological hypersensitivity to common substances found in the environment, such as house dust mites and pollens. The affected dogs groom excessively with licking or chewing of the paws, abdomen and perineum. The ears may be reddened and sore. With increasing pruritus, alopecia and redness of the skin become more evident and secondary yeast or bacterial infections are often seen. The causative allergens can be determined using an intradermal skin test (IDST) or serologically using an allergen-specific IgE-ELISA. In Hungary, IDST has been used to identify allergens in canine AD for more than 10 years, and serological tests have only become available within the past few years. However, for small animal practice it is important to know which test to choose and how to evaluate the results. The aim of the present study was to directly compare IDST with ELISA using a limited set of allergens.

Animals, Material and Methods

Animals

A total of 210 dogs with AD, aged between 1 and 10 years (94 females, 109 males and 7 spayed females), were included in the present study. The most common breeds were German shepherd dogs (18%), Hungarian vizslas (12%), boxers (9%) and West Highland white terriers (8%).

Dermatological examination

The diagnosis of AD was based on careful evaluation of the dog's clinical history and the presence of specific signs of disease. Three major and three minor criteria according to Willemse (1986) were established in all dogs. Skin scrapings for bacterial and fungal cultures were taken from all the pruritic dogs to eliminate the possibility of other pruritic skin diseases. To exclude *Sarcoptes* infestation, systemic scabidical therapy with Stronghold® spot on (Pfizer Inc. Animal Health) twice in a four-week interval was prescribed in all patients. Dogs with pyoderma, *Malassezia* infection, parasite infestation (fleas, *Cheyletiella* and *Sarcoptes*) and flea-allergic dermatitis were excluded from the study or treated with appropriate antibiotics, antimicrobics or antiparasitic drugs for a minimum of three weeks before IDST and serum collection.

Food elimination diet

All possible candidates were fed an eight-week elimination diet to exclude any adverse food reactions. The lamb, fish, rabbit, horse, venison or deer diet contained home-cooked protein which had never been given to the dog before and carbohydrate (potato or rice) boiled in salty wa-

ter. Only apples and carrots were additionally allowed. In the 210 dogs included in the study, the eight-week elimination diet was unsuccessful in curing the AD. Additional dogs with food allergy were excluded from the study.

Intradermal skin test

Before skin testing took place, oral and topical glucocorticoids were discontinued for at least three weeks, and antihistamine therapy was omitted for 10 days. IDST was performed on all 210 pruritic dogs. The allergen set for IDST (Artu Biologicals, Lelystad, Netherlands) contained the same allergens as those used for the ELISA (Allergopharma). Only single allergens were used for skin testing: house dust mites (*Dermatophagoideus farinae* and *Dermatophagoideus pteronyssinus*, meal mite (*Acarus siro*), copra mite (*Tyrophagus putrescentiae*), hay mite (*Lepidoglyphus destructor*), orchard grass (*Dactylis glomerata*), timothy (*Phleum pratense*), blue grass (*Poa pratensis*), ragweed (*Ambrosia elatior*), stinging nettle (*Urtica dioica*), plantain (*Plantago lanceolata*), mugwort (*Artemisia vulgaris*), and flea allergen.

Allergen-specific IgE, ELISA

Blood was collected from all animals using BD Vacutainer™ SST (Becton, Dickinson and Co.). The serum was immediately frozen and stored at -25°C until analysed by ELISA imovet-biocheck (Laboratory Laupeneck) using the D9 monoclonal antibody to detect allergen-specific canine IgE. To date, D9 is the only well-functioning monoclonal antibody against canine IgE validated by different independent researchers. With exception of a mixture of grass pollen, timothy grass (*Phleum pratense*), velvet grass (*Holcus lanatus*), cocksfoot (*Dactylis glomerata*), blue grass (*Poa pratensis*), meadow fescue (*Festuca pratensis*), ray grass (*Lolium perenne*) and a mixture of the tree pollen birch (*Betula pendula*), alder (*Alnus glutinosa*) and hazel (*Corylus avellana*) same selection of single allergens were used in the ELISA. Only the flea allergen whole body extract were from Greer. For evaluation of the ELISA, the results were compared with those of the IDST even though it is known that the IDST is not the “gold-standard” to determine the possible causative allergens of AD. Specificity, sensitivity, positive predicted value, negative predicted value and the accuracy were calculated (Tab 1).

Results

Assuming a correct diagnosis of AD according to the Willemse criteria and excluding sarcoptic mange, food allergy and flea-allergic dermatitis, 156 (74.3%) of the 210 atopic dogs had a positive IgE-ELISA result for at least one allergen tested, and 142 (67.6%) dogs were positive for IDST. The following antigens reacted in both the ELISA

and IDST: *D. farinae*, *A. siro*, *T. putrescentiae*, *ambrosia*, *L. destructor* and mugwort (Tab 2). With the exception of ambrosia and mugwort, pollen plays a less important role as a causative allergen in AD. Of the 210 atopic dogs, 132 (63.9%) were positive and 43 (20.5%) negative in both

the IgE-ELISA and IDST. Test results for all dogs diagnosed by ELISA and IDST are shown in Table 3. The most relevant information from these calculations is that the overall accuracy of the ELISA was 91.3% with a range of 81.4% to 99.5% depending on the specific allergens.

Table 1: Calculation of sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and accuracy (A) when comparing ELISA with IDST.

	IDST positive	IDST negative
ELISA positive	a	b
ELISA negative	c	d

$$Se = a / (a + c) \times 100\%$$

$$Sp = d / (b + d) \times 100\%$$

$$PPV = a / (a + b) \times 100\%$$

$$NPV = d / (c + d) \times 100\%$$

$$A = (a + d) / (a + b + c + d) \times 100\%$$

Table 2: Allergens and percentage of atopic dogs (n=210) that were positive for each allergen in ELISA and IDST.

Allergen	ELISA positive (%)	IDST positive (%)
<i>D. farinae</i>	53.1	52.6
<i>D. pteronyssinus</i>	3.3	1.9
<i>Acarus siro</i>	50.7	48.3
<i>Tyrophagus putr.</i>	60.8	44.5
<i>Lepidoglyphus destr.</i>	16.7	21.5
Grass mix	14.4	7.7
Ragweed	20.1	24.4
Plantain	8.6	2.4
Mugwort	18.7	16.7
Tree mix	10	4.3
Flea	11	10.5

Discussion

Besides the IDST which is normally used by specialised veterinarians, the commercial IgE-ELISA had not previously been assessed. It was important to investigate if the serological test could be used instead of the IDST.

The characteristic results of the skin test reaction in the current study are similar to those previously reported by others in USA and Europe (Sture et al., 1995; Reedy et al., 1997; Mueller et al., 2000; Shaw and Day, 2000; Saevik et al., 2003), with most common reactions being due to house dust mite allergens (except *D. pteronyssinus*) and only seldom due to pollen allergens. The exception among pollen is ragweed, an allergen quite commonly found in Hungary (Tarpataki et al., 2006). In USA, both house dust mites and pollen represent important allergen groups as well (DeBoer, 1989; Wassom and Grieve, 1998).

In the present study, the major allergens that tested positive in the ELISA were three species of house dust mites (*T. putrescentiae*, *D. farinae* and *A. siro*), ragweed, mugwort and tree pollen. The house dust mites *D. farinae* and *D. pteronyssinus* have been identified in several studies as major allergens in canine AD in Europe (Willemsse and Van Den Brom, 1983; Vollset, 1985; Sture et al., 1995; Saridomichelakis et al., 1999; Tarpataki et al., 2006) and in USA (Scott et al., 2001).

Overall sensitivity, specificity and predictive value

The ELISA in the present study shows an acceptable overall sensitivity of 82.4% when compared with the IDST.

Table 3: Test results of all dogs (n=210) using ELISA and IDST.

	a	b	c	d	Se (%)	Sp (%)	PPV (%)	NPV (%)	A (%)
<i>D. farinae</i>	94	15	16	85	85.4	85	86.2	84.2	85.2
<i>D. pteronyssinus</i>	0	6	4	100	0	97.1	0	98	95.2
<i>Acarus siro</i>	84	13	16	97	84	88.2	86.6	85.8	86.2
<i>Tyrophagus putr.</i>	86	29	10	85	88.7	74.6	75.8	89.5	81.4
<i>Lepidoglyphus</i>	33	1	11	165	75	99.4	97.1	93.8	94.3
Grass mix	10	13	5	182	66.7	93.3	43.5	97.3	91.4
Ragweed	36	5	14	155	72	96.9	87.8	91.7	90.9
Plantain	4	10	1	195	80	95.1	28.6	99.5	94.8
Mugwort	34	1	0	175	100	99.4	97.1	100	99.5
Tree mix	7	11	1	191	87.5	95	38.9	99.5	94.3
Flea	10	3	7	190	58.8	98.4	76.9	96.4	95.2
Total	398	107	85	1620	82.4	93.8	78.8	95.1	91.3

a=positive ELISA and positive IDST, b=positive ELISA and negative IDST, c=positive ELISA and negative IDST,

d=negative ELISA and negative IDST, Se=sensitivity, Sp=specificity, PPV=positive predictive value, NPV=negative predictive value, A=accuracy.

120 Originalarbeiten

Considering with other ELISA evaluated in earlier reports (Miller et al., 1992; Codner and Lessard 1993; Bond et al., 1994; Bevier et al., 1997; Mueller, 1999), the ELISA employed in the current study exhibits an overall sensitivity of 82.4%, which is higher than in the studies by Saevik (2003) with 53.6% and Ginel et al., (1998) and 72.2% respectively. So far only Mueller et al. (1999) have reported a higher sensitivity (90.4%).

The ELISA movet/biocheck shows an excellent overall specificity of 93.8% compared with earlier evaluations of ELISA from competitors with overall specificities of 41.6% (Ginel et al., 1998), 91.6% (Mueller et al., 1999), 84.4% (Sævik, 2003) and 0% (Codner and Lessar, 1993). The overall positive predictive value (78.8%) of the ELISA compared with the IDST was not as high as for the overall negative predictive value (95.1%). The chance of getting the same result with ELISA as with IDST was 91.3%.

Sensitivity and specificity of single allergens

By looking at a selection of allergens more details about the value of ELISA can be seen. The sensitivity of detecting IgE directed against *D. farinae* allergens by ELISA was lower in the present study (85.4%) than in previous reports (Lian, 1998 [92.5%], Mueller, 1999 [95.1%]), but the specificity (85%) was higher than reported by Lian (1998 [(44.4%)]) but slightly lower (96.3%) than reported by Mueller et al., (1999). The sensitivity of the IgE-ELISA directed against *D. pteronyssinus* allergens was 0%, probably due to the fact that only 6 ELISA and 4 IDST positive cases were found among the 210 atopic dogs. This could be due to the low prevalence of *D. pteronyssinus* in Hungary, or that the allergens were from different sources and were not recognised by corresponding IgE antibodies. However, the specificity was much higher (97.1%) compared to the study of Lian (1998 [29.4%]).

The ELISA-based sensitivities for the detection of IgE antibodies directed against flea antigens are reported to be very variable, with values of 78% (McCall et al., 1997) and 50% (Lian, 1998). In the present study, a sensitivity of 58.8% and a specificity of 98.4% was achieved. Others have reported specificities of 49.4% (Lian 1998) and 91% (McCall et al., 1997) using competitors' ELISAs. As in our investigation dogs with typical flea-allergy dermatitis were also eliminated in the studies mentioned above because dogs with flea infestations have to be validated very carefully. Using dogs with flea-allergic dermatitis would probably increase the number of positive ELISA and IDST and change the sensitivity calculation results. On the other hand the diagnostic evaluation of dogs with allergic reactions towards flea allergens depends on the source of the flea allergens used in the test. As recombinant flea allergens Cte f1 and Cte f2 are not commercially available, whole-body extracts had to be used. The same source of allergen extracts was not used in both the ELISA and IDST, so the results could be different.

Predictive value and accuracy of single allergens

The results of positive and negative predictive values in the most common and positive by reacted allergens, (*D. farinae*, *A. siro*, *Lepidoglyphus* and *Ambrosia*,) were satisfactory (positive predictive value range 86.2%–97.1%, negative predictive value range 84.2%–100%) with the exception of *T. putrescentiae*, where the positive predictive value was slightly lower (75.8%) than the overall average of the positive predictive value of all allergens (78.8%). In some allergens (low prevalence, allergen extracts of different sources) such as *D. pteronyssinus*, tree pollens, grass mix and plantain, the positive predictive value was low (range 0%–43.5%).

The value of accuracy of each allergen shows the percentage of the chance of getting the same results (positive or negative) with the IDST as well as with ELISA. The accuracy range between 81.4% and 99.5% shows that the ELISA is a very reliable test when compared with the IDST.

Comparing clinical diagnosis of AD with results of IDST and allergen-specific IgE

All the atopic dogs (n = 210) in the present study were considered to fulfil the criteria for a diagnosis of AD, but 32.4% (68 of 210) were still IDST negative. It is difficult to explain the likelihood that false-negative IDST results occurred as there is no means of determining the accuracy of the IDST. Only with a provocative allergen challenge of the dog could the possibility of a false-negative IDST be shown. DeBoer (1989) reported up to 20% and Foster (2003) up to 16% negative IDST results in suspected AD. Hillier and DeBoer (2001) reviewed the factors that may lead to false-negative results, which included drug interference and inherent host factors. However, IDST is still considered useful and it represents the preferred diagnostic technique (Willemse and Van Den Brom, 1983; Reedy et al., 1997; Scott et al., 2001).

Skin testing and serological assays measure different subsets of the IgE response to an antigen. The IDST demonstrates IgE bound to the mast cell via Fc RI. The half-life of these antibodies varies. For example, in humans, mast cell-bound IgE has a half-life of up to 14 days compared with 2.3 days for serum IgE (Ishizaka and Ishizaka, 1975). IDST may remain positive for several months after serum levels of allergen-reactive IgE have waned, most likely because the remaining antibodies are bound to the surface of mast cells in tissues rather than in blood circulation (Wassom et al., 1998). Also there may be different types of canine IgE that exhibit different biological properties (Halliwell et al., 1998; Lian and Halliwell, 1998; Scott et al., 2001; Hillier and DeBoer, 2001). Another factor to be considered is that the allergens used for the IDST and ELISA were from two different companies. Consequently, it is not surprising that the results of a serological assay do not always correlate with those of the IDST.

Conclusions

In practice, the evaluated IgE-ELISA seems as reliable for the detection of possible causative allergens in AD as the IDST, even if the allergen preparations used in the two tests were not identical. The overall values of specificity, sensitivity, positive and negative predictive values and accuracy provide good comparison between ELISA and IDST.

Dermatite atopique du chien: comparaison entre test intra cutané et IgE spécifique d'allergènes

Le but de la présente étude était de comparer, chez 210 chiens souffrant de dermatite atopique, les résultats d'un test intra cutané (TIC) avec ceux d'une recherche par ELISA des IgE spécifiques d'allergènes. Chez 210 chiens souffrant cliniquement d'une dermatite atopique, un TIC a été réalisé. Les sérums de ces chiens ont été soumis à un test ELISA pour mettre en évidence les IgE spécifiques d'allergènes en utilisant un anticorps monoclonal D9 dirigé contre l'IgE canine. Dans cette comparaison, les valeurs des TIC ont été considérées comme le standard. Dans les deux tests, on a obtenu des réactions positives aux allergènes suivants: *Dermatophagoides farinae*, *Acarus siro*, *Tyrophagus putrescentiae*, ambrosie, artemisia, et *Lepidoglyphus destructor*. Le test ELISA montrait une sensibilité de 82,4% et une spécificité de 93,8%. L'exactitude pour tous les prélèvements positifs et négatifs s'élevait à 91,3%. L'utilisation de l'anticorps monoclonal D9 dans un test ELISA se montre fiable pour mesurer les IgE spécifiques d'allergènes dirigés contre des produits qui peuvent déclencher une dermatite atopique. La détermination des allergènes est particulièrement utile lorsqu'on envisage une désensibilisation.

In the present study we could show that in the absence of IDST ELISA can be used to determine the causative allergens provoking AD.

On the other hand it is important to know that the diagnosis of AD has to be performed clinically and not only by IDST or ELISA measuring allergen specific IgE. Results of both tests can only be used to determine allergens that should be avoided to control clinical symptoms within appropriate therapeutic measures or for hyposensitisation.

Dermatite atopica dei cani: paragone tra test intracutaneo e di uno specifico allergene IgE

Scopo di questo studio era di confrontare in 210 cani affetti da dermatite atopica i risultati del test intracutaneo con quello di uno specifico allergene IgE-ELISA. In 210 cani con una diagnosi clinica di una dermatite atopica è stato effettuato un test intracutaneo. I sieri relativi sono stati esaminati tramite ELISA su uno specifico allergene IgE, dove l'anticorpo monoclonale D9 viene utilizzato contro la IgE canine. In questo confronto i valori del test intracutaneo sono stati presi quali standard. In entrambi i test sono state raggiunte delle reazioni positive con i seguenti allergeni: *Dermatophagoides farinae*, *Acarus siro*, *Tyrophagus putrescentiae*, ambrosia, artemisia e *Lepidoglyphus destructor*. Il test ELISA ha avuto in totale una sensibilità dell'82,4% e una specificità del 93,8%. La precisione per tutti i campioni positive e negativi raggiungeva il 91,3%. L'uso dell'anticorpo monoclonale D9 si è dimostrato nel test ELISA un affidabile rilevatore di anticorpi per la misurazione di specifici allergeni IgE del cane contro gli allergeni che potevano provocare una dermatite atopica. La determinazione dell'allergene che fa insorgere la malattia è in particolare di aiuto se si desidera una desensibilizzazione.

References

Bevier D. E., Mondesire R. L., Rose B. J., Wassom D. L.: Fc 1-based ELISA technology for in vitro determination of allergen-specific IgE in a population of intradermal skin-tested normal and atopic dogs. In: Clinical Advances: A Supplement to Compend. Contin. Educ. Vet. Pract. 1997, 3: 10–16.

Bond R., Thorogood S. C., Lloyd D. H.: Evaluation of two enzyme-linked immunosorbent assays for the diagnosis of canine atopy. Vet. Rec. 1994, 135: 130–133.

DeBoer D. J.: Survey of intradermal skin testing practices in North America. J. Am. Vet. Med. Assoc. 1989, 195: 1357–1363.

DeBoer D. J.: Canine Atopic Dermatitis: New Targets, New Therapies. WALTHAM International Science Symposium: Nature, Nurture, and the Case for Nutrition. Supplement of J. Nutr. 2004, 134: 2056S–2061S.

Codner E. C., Lessard P.: Comparison of intradermal allergy test and enzyme-linked immunosorbent assay in dogs with allergic skin disease. J. Am. Vet. Med. Assoc. 1993, 202: 739–743.

Foster A. P., Littlewood J. D., Webb P., Wood J. L., Rogers K., Shaw S. E.: Comparison of intradermal and serum testing for allergen-specific IgE a FcRI-based assay in atopic dogs in the UK. Vet. Immunol. Immunopathol. 2003, 93: 51–60.

122 Originalarbeiten

- Ginel P. J., Riaño C., Lucena R.:* Evaluation of a commercial ELISA test for the detection of allergen-specific IgE antibodies in atopic dogs. *Zentralbl. Veterinarmed. B.* 1998, 45: 421–425.
- Halliwell R. E. W., Gilbert S. M. and Lian T. M.:* Induced and spontaneous IgE antibodies to *Dermatophagoides farinae* in dogs and cats: evidence of functional heterogeneity of IgE. *Vet. Dermatol.* 1998, 9: 179–184.
- Hillier A. and DeBoer D. J.:* The ACVD Task Force on Canine Atopic Dermatitis (XVII): intradermal testing. *Vet. Immunol. Immunopathol.* 2001, 81: 289–304.
- Lian T. M., Halliwell R. E. W.:* Allergen-specific IgE and IgGd antibodies in atopic and normal dogs. *Vet. Immunol. Immunopathol.* 1998, 66: 203–223.
- McCall C. A., Steadman K. E., Penner S. J.:* Fc 1 -based ELISA measurement of anti-flea saliva IgE in dogs. In: *Clinical Advances: A Supplement to Compendium on Compend. Contin. Educ. Vet. Pract.* 1997, 3: 24–28.
- Miller W. H., Scott D. W., Scarlett J. M.:* Evaluation of an allergy screening test for use in atopic dogs. *J. Am. Vet. Med. Assoc.* 1992, 200: 931–935.
- Mueller RS., Burrows A. and Tsohalis J.:* Comparison of intradermal testing and serum testing for allergen-specific IgE using monoclonal IgE antibodies in 84 atopic dogs. *Aust. Vet. J.* 1999, 77: 290–294.
- Mueller R. S., Bettenay S. V., Tideman L.:* Aero-allergens in canine atopic dermatitis in southeastern Australia based on 1000 intradermal skin tests. *Aust. Vet. J.* 2000, 78: 392–399.
- Reedy L. M., Miller W. H., Willemse T.:* Allergic Skin Diseases of Dogs and Cats. W.B. Saunders, Philadelphia. 1997, 67–98.
- Saridomichelakis M. N., Koutinas A. F., Gioulekas D., Leontidis L.:* Canine atopic dermatitis in Greece: clinical observation and the prevalence of positive intradermal test reactions in 91 spontaneous cases. *Vet. Immunol. Immunopathol.* 1999, 69: 61–73.
- Sævik B. K., Ulstein T. L., Larsen H. J. S.:* Evaluation of a commercially available enzyme-linked immunosorbent assay for the detection of allergen-specific IgE antibodies in dogs. *Res. Vet. Sci.* 2003, 74: 37–45.
- Scott D. W., Miller W. H., Griffin C. E.:* *Small Animal Dermatology*, WB Saunders, Philadelphia. 2001, 543–666.
- Shaw S. E., Day M. J.:* Recent developments in atopic dermatitis of companion animals In: Williams HC (Ed), *The Epidemiology of Atopic Dermatitis*. Cambridge University Press, Cambridge. 2000, 233–244.
- Sture G. H., Halliwell R. E. W., Thoday K. L., van den Broek A. H., Henfrey J. I., Lloyd D.H., Mason I.S., Ferguson E.:* Canine atopic disease: the prevalence of positive intradermal skin tests at two sites in the north and south of Great Britain. *Vet. Immunol. Immunopathol.* 1995, 44: 293–308.
- Tarpataki N., Pápa K., Reiczigel J., Vajdovich P., Vörös K.:* Prevalence and features of canine atopic dermatitis in Hungary. *Acta. Vet. Hung.* 2006, 54: (in press).
- Vollset I.:* Atopic dermatitis in Norwegian dogs. *Nordisk Veterinærmedicin.* 1985, 37: 97–106.
- Wassom D. L. and Grieve R. B.:* In vitro measurement of canine and feline IgE: a review of Fc RI -based assays for detection of allergen-reactive IgE. *Vet. Dermatol.* 1998, 9: 173–178.
- Willemse T, Van Den Brom W. E.:* Investigations of the symptomatology and the significance immediate skin test reactivity in canine atopic dermatitis. *Res. Vet. Sci.* 1983, 34: 261–265.
- Willemse T.:* Atopic skin disease: a review and a consideration of diagnostic criteria. *J. Small. Anim. Pract.* 1986, 27: 771–778.

Corresponding author

Beat Bigler, Labor Laupeneck/ imovet bg
Laupenstrasse 33 CH-3008, Bern, Switzerland
Telephone: +41-31-381-5959
Fax: +41-31-381-3414
E-mail: bigler@bluewin.ch

Received: 16 May 2007

Accepted: 14 September 2007