

## Comparison of Intradermal Tests, Total Serum IgE Concentrations and Allergen-Specific IgE Using an Arrayed Protein Chip in Atopic Dogs

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**Abstract:** Canine atopic dermatitis is a common pruritic skin disease which is most commonly associated with Immunoglobulin (Ig) E immune response to environmental allergens. The aims of this study were to report comparison between total serum IgE concentrations in atopic and non-atopic dogs. Comparison between results of intradermal tests and serum allergen-specific IgE arrayed protein chips was also investigated. Total serum IgE concentrations between 37 atopic dogs ( $92.5 \pm 102.3 \mu\text{g mL}^{-1}$ ) and 34 non-atopic dogs ( $16.2 \pm 30.2 \mu\text{g mL}^{-1}$ ) were significantly different ( $p < 0.001$ ). A positive correlation between total serum IgE concentrations and corresponding modified canine atopic dermatitis extent and severity index scores was found ( $r = 0.56$ ,  $p < 0.001$ ) in atopic dogs. The cut-off value was  $16.8 \mu\text{g mL}^{-1}$  with the sensitivity of 91.9% and the specificity of 85.3%. The overall sensitivity of specific IgE serum tests was 24%, specificity was 95% and efficacy was 81%. Total serum IgE concentrations could be a screening test and determine severity of atopic dermatitis in dogs with discrete clinical evaluation. However, arrayed protein chip method might not be suitable for screening functional allergen-specific IgE.

**Key words:** Atopic dermatitis, arrayed protein chip, dog, IgE, serum, screening

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### INTRODUCTION

Canine atopic dermatitis is a genetically predisposed inflammatory skin disease which is most commonly associated with Immunoglobulin (Ig) E immune response to environmental allergens (DeBoer and Hillier, 2001b). This condition is characterized by pruritus, mainly affecting the ears, eyes, distal extremities and trunk with a resultant of otitis externa, conjunctivitis and pododermatitis (Scott *et al.*, 2001; Favrot *et al.*, 2010).

Diagnoses of atopic diseases of dogs are strongly dependent on acquiring accurate medical histories and assessing the corresponding clinical signs including a thorough differential diagnostic process that rules out other (non-related) complications (Willemse, 1986; Prelaud *et al.*, 1998; DeBoer and Hillier, 2001a; Hillier and DeBoer, 2001; Scott *et al.*, 2001; Favrot *et al.*, 2010). In addition, severity of clinical signs associated with atopic dermatitis in dogs can also be semi-quantitated using modified a Canine Atopic Dermatitis Extent and Severity Index (CADESI) (Olivry *et al.*, 2007, 2008). Once the clinical diagnosis of atopic dermatitis has been made allergy testing can be performed to identify the offending allergens. This can be achieved using intradermal allergen tests, atopic patch tests or *in vitro* serologic allergy tests

(Mueller *et al.*, 1999; DeBoer and Hillier, 2001b; Foster *et al.*, 2003; Olivry *et al.*, 2005). Subsequent selecting offending allergens for immunotherapy is also based on a complete reliable allergen test (Park *et al.*, 2000).

Increasing total serum IgE concentrations represents a major empiric risk factor for development of atopic disease and are used as a diagnostic indicator of atopic dermatitis in human patients (Berg and Johansson, 1969; Juhlin *et al.*, 1969) whereas diagnostic value of IgE concentrations remains controversial in canine atopic dermatitis (Wilkie *et al.*, 1990; Hill *et al.*, 1995; DeBoer and Hill, 1999; Roque *et al.*, 2011). The aims of this study were firstly to quantitate total serum IgE concentrations in atopic and non-atopic dogs; relationship between total serum IgE concentrations and a modified CADESI in atopic dogs was also evaluated. Secondly, correlation between Intra Dermal Tests (IDT) and allergen-specific IgE was also investigated.

### MATERIALS AND METHODS

**Animals:** Seventy one dogs admitted to the Dermatological Clinic at the National Taiwan University Veterinary Hospital during 2008 to 2009 were enrolled for

Table 1: The breeds and numbers of 37 atopic dogs and 34 non-atopic dogs represented in the study

Breeds	Atopic (n = 37)	Non-atopic (n = 34)	Total (n = 71)
Akita inu	0	2	2
Beagle	2	2	4
Bichon frise	1	0	1
Boston terrier	1	0	1
Chihuahua	2	3	5
English bulldog	2	0	2
Golden retriever	4	3	7
Husky	2	0	2
Labrador retriever	2	2	4
Maltese terrier	4	4	8
Miniature dachshund	3	1	4
Miniature schnauzer	2	3	5
Pomeranian	0	3	3
Pug	0	1	1
Shiba inu	2	0	2
Shih Tzu	1	1	2
Toy Poodle	1	3	4
Pembroke Welsh Corgi	1	0	1
West Highland terrier	2	0	2
Mix breed	5	5	10

this study; 37 dogs were categorized as atopic group (23 were males, 14 were females with a mean age of 5.2±3.3 years); 34 dogs that were free from any signs of skin problems were included as a group of non-atopic group (19 were males, 15 were females with a mean age of 8.6±4.2 years). Breeds represented in this study are shown in Table 1. All dogs in this study were regularly medicated with heartworm preventatives, ectoparasitic and endoparasitic preventatives monthly.

**Inclusion criteria of atopic dermatitis:** Inclusion criteria for atopic disease in this study included clinical signs based upon the fulfillment of Willemse’s clinical criteria (Willemse, 1986). Flea hypersensitivity and ectoparasites infection were ruled out by means of standard diagnostic method. All atopic dogs were free from any forms of corticosteroids and cyclosporine at least for 4 weeks. Fatty acid supplements and non-medicated shampoos or humectants were allowed.

A modified CADESI (Olivry *et al.*, 2007) was applied and scored in all dogs at the time of enrollment. In this modified version, three degrees of severity of clinical signs (erythema, excoriations, papules/pustules, lichenification, self-induced alopecia) was assessed of 40 sites on the body. In this modified version, the highest score theoretically was the maximum CADESI score was 40×5×3 = 600.

**Blood sample collection:** Blood samples were taken by venipuncture and coagulated in upright polypropylene tubes in room temperature for 1 h. The samples were then

centrifuged at 3000 g for 10 min. The serum was obtained and placed into an aliquotand then stored at -20°C until the time of assay.

**Evaluation of total serum immunoglobulin E concentrations using Enzyme-Linked Immuno Sorbent Assay (ELISA):** Total serum IgE was quantified using a commercial kit and according to manufacturer’s instructions (Bethyl Laboratories, USA). Briefly, microplates were coated with 1 mg mL<sup>-1</sup> goat anti-dog IgE purified antibody diluted in 100 mL carbonate-bicarbonate buffer at pH 9.6 and apply 100 µL to each well. Phosphate-Buffered Saline (PBS, pH 7.4) with 0.05% Tween 20 (PBS-Tween) containing 1% BSA was used as the blocking buffer and applied for 30 min at 37°C. Serum samples were diluted 1:1000 in blocking buffer and incubated for 1 h at room temperature.

Goat anti-dog IgE conjugated with horseradish peroxidase diluted 1:10000 was used as an indicator. After incubation for 1 h at room temperature, the reactions were visualized by the addition of the enzyme substrate tetramethyl benzidine for 30 min and apply 100 µL 2 M sodium di-hydrogen orthophosphate to stop the reaction, wavelength for tetramethyl benzidine should be 450 nm (Bio-Tek Instruments Inc., USA).

**Allergen-specific IgE using arrayed protein chip:** Allergens of *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, human dander, cat epithelia, German cockroach and *Acacia* sp. were purified and spotted duplicately on each chip (Chu *et al.*, 2004), canine IgE and actin were also spotted on each chip as positive and negative controls, respectively. The allergen-arrayed proteins chips were immersed for 1 h in 80 µL of the canine serum. The allergen chips were then washed twice with PBS incubated with anti-canine IgE-Cy3 for 1 h were washed twice with PBS and then washed twice with double deionized water at the end of incubation. The reacted chips were scanned by a GenePix 4000B scanner (Axon Instrument Inc, California, USA) to detect fluorescence intensities generated by Cy3-conjugated anti-canine IgE.

**Intradermal allergen tests:** The majority dogs included in this study were small breeds and the skin surface for IDT was very limited, selection of allergens was based on the most common local allergens in atopic dogs: *D. farinae*, *D. pteronyssinus*, human dander, cat epithelia, German cockroach and *Acacia* sp. were included in this study. Results were evaluated after 15 min by assessing the diameter of wheal with a 4 point scale (0, 1+, 2+, 3+ and 4+). Reactions that were ≥2+ were considered positive.

**Comparison between allergen-specific IgE and intradermal allergen tests:** The allergens that were compared between the two tests were *D. farinae*, *D. pteronyssinus*, human dander, cat epithelia, German cockroach and *Acacia* sp.

**Statistics:** All statistical analysis was performed using the Statistical Software (Graph Pad Prism 4.0 for Windows, USA). Values of all data were expressed as mean± Standard Deviation (SD). The Mann Whitney test was used for comparison the effect of gender and the difference between total IgE concentrations in atopic and non-atopic dogs. The correlation between total IgE concentrations and age in both groups and CADESI scores in atopic dogs were evaluated by the Spearman's rank test. The reliability of total serum IgE concentrations in diagnosis of AD was assessed by examining the sensitivity and the specificity of the cut-off value. The results of allergen-specific IgE were compared to the results of IDT. All tests were designed as two-tailed test. The  $p < 0.05$  was considered statistically significant. Using the IDT as the standard, sensitivity, specificity and efficacy of specific-IgE detected by allergen protein chip were calculated according to standard formulas.

**RESULTS AND DISCUSSION**

**Association between non-atopic dogs, dogs with atopic dermatitis and total serum IgE concentration:** The most frequent lesions recorded in atopic dogs including various degree of pruritus and self-induced alopecia on periocular, perilabial, lateral pinna, metacarpal and tarsal areas.

The total serum IgE concentrations of non-atopic dogs ( $16.2 \pm 30.2 \mu\text{g mL}^{-1}$ ) and atopic dogs ( $92.5 \pm 102.3 \mu\text{g mL}^{-1}$ ) and were significantly different ( $p < 0.001$ , Fig. 1). A significant correlation ( $r = 0.56$ ,  $p < 0.001$ ) was found between the modified CADESI and total serum IgE concentrations in atopic dogs (Fig. 2).

The cut-off value was set at  $16.8 \mu\text{g mL}^{-1}$  with the sensitivity of 91.9% and the specificity of 85.3%. The Receiver-Operating Characteristic (ROC) curve was represented in Fig. 3. The total serum IgE concentrations were not affected by gender in non-atopic dogs ( $p = 0.19$ ) or in atopic dogs ( $p = 0.28$ ). No correlation was found between age and total serum IgE concentrations in either non-atopic dogs ( $r = 0.24$ ,  $p = 0.18$ ) or in atopic dogs ( $r = 0.01$ ,  $p = 0.93$ ).

**Comparison between Allergen-Arrayed Chips specific-IgE tests and results of IDST:** The sensitivity,

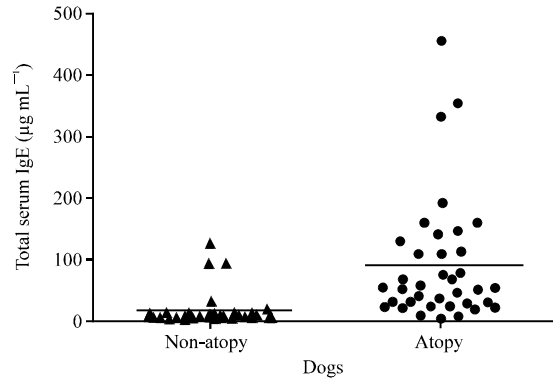


Fig. 1: Scatter plot of total serum IgE concentrations in 34 non-atopic and 37 atopic dogs, bars indicate mean

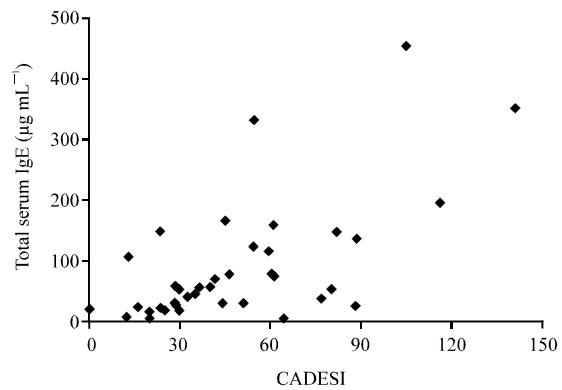


Fig. 2: Total serum IgE concentrations of atopic dogs in relation to their Canine Atopic Dermatitis Extent and Severity Index (CADESI,  $r = 0.56$ ,  $p < 0.001$ )

Table 2: Sensitivity, specificity and efficacy of allergen specific-IgE comparing to intradermal test

Allergen-specific IgE	Sensitivity (%)	Specificity (%)	Efficacy (%)
<i>D. farinae</i>	22	94	70
<i>D. pteronyssinus</i>	11	100	70
Human dander	33	100	93
Cat epithelia	50	100	96
German cockroach	25	96	85
<i>Acacia</i> sp.	29	89	70

specificity and efficacy was varied in among tested allergens (Table 2). The overall sensitivity of allergen-arrayed chip was 24% (ranged 11-50%), specificity was 95% (ranged 89-100%) and efficacy was 81% (ranged 70-96%).

Over these 2 decades, clinical value of total serum IgE concentrations in discrimination of atopic dermatitis in dogs remained controversial, range of concentrations was overlapped between atopic and non-atopic dogs (Halliwell and Kunkle, 1978; Hill and DeBoer, 1994;

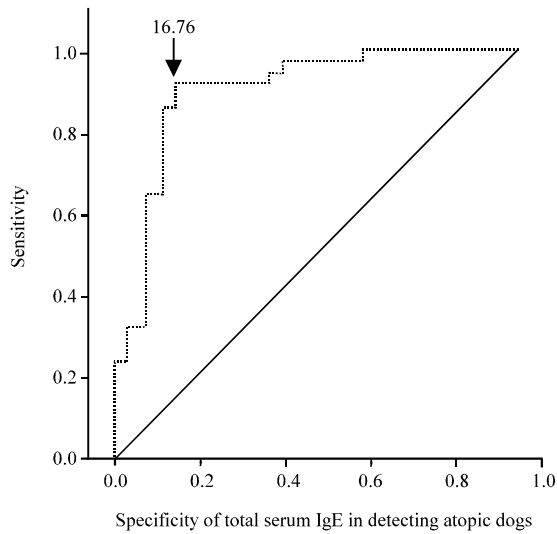


Fig. 3: Receiver-Operating Characteristic (ROC) curve. The cut-off value of total serum IgE concentrations between non-atopic and atopic dogs was set at  $16.8 \mu\text{g mL}^{-1}$

Hammerling and De Weck, 1998; Zunic, 1998; Park *et al.*, 2000; Foster *et al.*, 2003; Saevik *et al.*, 2003; Olivry *et al.*, 2005; Lee *et al.*, 2009). Total serum IgE concentrations were affected by many factors such as breeds, age, sex and status of parasite infestation (Hill *et al.*, 1995; DeBoer and Hill, 1999; Griot-Wenk *et al.*, 1999; Racine *et al.*, 1999; Fraser *et al.*, 2003; Roque *et al.*, 2011). However, relationship between total serum IgE concentrations and CADESI has not been reported in earlier studies.

In this study, total serum IgE concentrations were significantly different between atopic and non-atopic dogs. A positive correlation between total serum IgE concentrations and CADESI was also demonstrated. These findings indicated that total serum IgE concentrations may be applied as a screen test for atopic dermatitis in dogs. Together with discretely clinical evaluation, concentrations of total serum IgE may also determine the severity of atopic dermatitis.

The correlation between the allergen-specific IgE and intradermal allergen test was not satisfied in this study with low sensitivity, high specificity and moderate efficacy. These findings suggested that the method of arrayed protein chip was not detecting functional allergen-specific IgE. Similar results, a low sensitivity (ranged 19.3-100%) and a relatively high specificity (ranged 35-100%) were also reported in other studies using different methods such as Enzyme-Linked Immunosorbent Assay (ELISA), monoclonal antibody cocktail-based ELISA, high affinity-IgE receptor-based

ELISA, Fc-epsilon receptor alpha-chain protein-based assay and immunodot assay, etc. (Hammerling and De Weck, 1998; Zunic, 1998; Mueller *et al.*, 1999; DeBoer and Hillier, 2001b; Foster *et al.*, 2003; Saevik *et al.*, 2003). The source of allergens, allergen extraction process, reacting phase of allergens, IgE-specific detection reagent and signal molecule can also affect results of tests (Meyer *et al.*, 1994; Patel *et al.*, 1995; Hammerling and De Weck, 1998; Mari *et al.*, 1999; Stedman *et al.*, 2001; Olivry *et al.*, 2005; Lee *et al.*, 2009).

In the past, IDT has been the favored method for immediate demonstrating the presence of IgE for diagnosis of atopic dermatitis in dogs. Subsequent selection of offending allergens for immunotherapy is also completely based on the results of the test (Park *et al.*, 2000). Nevertheless, availability of skin surface area in small breeds of dogs for number of tested allergens and lacking familiarity of techniques and procedures limits the clinical application of intradermal allergen test in general practices. Identification of offending allergens in small breed dogs or dogs with limited skin surface for IDT and subsequent immunotherapy based on reliable methods *in vitro* is highly expected. Improvement of arrayed protein chip in identification of offending allergens is warranted.

## CONCLUSION

Total serum IgE concentrations could be a screening test and determine severity of atopic dermatitis in dogs with discrete clinical evaluation. However, Arrayed Protein Chip Method might not be suitable for screening functional allergen-specific IgE.

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## REFERENCES

- Berg, T. and S.G. Johansson, 1969. IgE concentrations in children with atopic diseases: A clinical study. *Int. Arch. Allergy*, 36: 219-232.
- Chu, S.L., K.H. Chen, C.Y. Tsai, C.M. Huang and C.H. Hsu, 2004. Efficacy of allergen-arrayed chips for detecting allergen-specific immunoglobulin E in allergic patients. *Mid. Taiwan J. Med.*, 9: 96-102.

- DeBoer, D.J. and A. Hillier, 2001a. The ACVD task force on canine atopic dermatitis (XV): Fundamental concepts in clinical diagnosis. *Vet. Immunol. Immunop.*, 81: 271-276.
- DeBoer, D.J. and A. Hillier, 2001b. The ACVD task force on canine atopic dermatitis (XVI): Laboratory evaluation of dogs with atopic dermatitis with serum-based "allergy" tests. *Vet. Immunol. Immunop.*, 81: 277-287.
- DeBoer, D.J. and P.B. Hill, 1999. Serum immunoglobulin E in West Highland White Terrier puppies do not predict development of atopic dermatitis. *Vet. Dermatol.*, 10: 275-281.
- Favrot, C., J. Steffant, W. Seewald and F. Picco, 2010. A prospective study on the clinical features of chronic canine atopic dermatitis and its diagnosis. *Vet. Dermatol.*, 21: 23-31.
- Foster, A.P., J.D. Littlewood, P. Webb, J.L. Wood, K. Rogers and S.E. Shaw, 2003. Comparison of intradermal and serum testing for allergen-specific IgE using a Fcepsilon RI $\alpha$ -based assay in atopic dogs in the UK. *Vet. Immunol. Immunop.* 93: 51-60.
- Fraser, M.A., P.E. McNeil and G. Gettinby, 2003. Studies of total serum immunoglobulin E concentrations in atopic and non-atopic dogs. *Vet. Rec.*, 152: 159-163.
- Griot-Wenk, M.E., A. Busato, M. Welle, B.P. Racine, R. Weilenmann, P. Tschud and A. Tipold, 1999. Total serum IgE and IgA antibody levels in healthy dogs of different breeds and exposed to different environments. *Res. Vet. Sci.*, 67: 239-243.
- Halliwell, R.E. and G.A. Kunkle, 1978. The radioallergosorbent test in the diagnosis of canine atopic disease. *J. Allergy Clin. Immunol.*, 62: 236-242.
- Hammerling, R. and A.L. De Weck, 1998. Comparison of two diagnostic tests for canine atopy using monoclonal anti-IgE antibodies. *Vet. Dermatol.*, 9: 191-199.
- Hill, P.B. and D.J. DeBoer, 1994. Quantification of total serum IgE concentration in dogs by use of an enzyme-linked immunosorbent assay containing monoclonal murine anti-canine IgE. *Am. J. Vet. Res.*, 55: 944-948.
- Hill, P.B., K.A. Moriello and D.J. DeBoer, 1995. Concentrations of total serum IgE, IgA and IgG in atopic and parasitized dogs. *Vet. Immunol. Immunopathol.*, 44: 105-113.
- Hillier, A. and D.J. DeBoer, 2001. The ACVD task force on canine atopic dermatitis (XVII): Intradermal testing. *Vet. Immunol. Immunopathol.*, 81: 289-304.
- Juhlin, L., G.O. Johansson, H. Bennich, C. Hogman and N. Thyresson, 1969. Immunoglobulin E in dermatoses: Levels in atopic dermatitis and urticaria. *Arch. Dermatol.*, 100: 12-16.
- Lee, K.W., K.D. Blankenship, Z.M. McCurry, R.E. Esch, D.J. DeBoer and R. Marsella, 2009. Performance characteristics of a monoclonal antibody cocktail-based ELISA for detection of allergen-specific IgE in dogs and comparison with a high affinity IgE receptor-based ELISA. *Vet. Dermatol.*, 20: 157-164.
- Mari, A., P. Iacovacci, C. Afferni, B. Barletta, R. Tinghino, G. Di Felice and C. Pini, 1999. Specific IgE to cross-reactive carbohydrate determinants strongly affect the *In vitro* diagnosis of allergic diseases. *J. Allergy Clin. Immunol.*, 103: 1005-1011.
- Meyer, C.H., J.F. Bond, M.S. Chen and M.T. Kasaian, 1994. Comparison of the levels of the major allergens Der p I and Der p II in standardized extracts of the house dust mite, *Dermatophagoides pteronyssinus*. *Clin. Exp. Allergy*, 24: 1041-1048.
- Mueller, R.S., A. Burrows and J. Tsohalis, 1999. Comparison of intradermal testing and serum testing for allergen-specific IgE using monoclonal IgE antibodies in 84 atopic dogs. *Aust. Vet. J.*, 77: 290-294.
- Olivry, T., H.A. Jackson, K.M. Murphy, K.C. Tater and M. Roberts, 2005. Evaluation of a point-of-care immunodot assay for predicting results of allergen-specific intradermal and immunoglobulin E serological tests. *Vet. Dermatol.*, 16: 117-120.
- Olivry, T., R. Marsella, T. Iwasaki, R. Mueller and ITFCAD, 2007. Validation of CADESI-03, a severity scale for clinical trials enrolling dogs with atopic dermatitis. *Vet. Dermatol.*, 18: 78-86.
- Olivry, T., R. Mueller, T. Nuttall, C. Favrot, P. Prelaud and ITFCAD, 2008. Determination of CADESI-03 thresholds for increasing severity level of canine atopic dermatitis. *Vet. Dermatol.*, 19: 115-119.
- Park, S., F. Ohya, K. Yamashita, K. Nishifuji and T. Iwasaki, 2000. Comparison of response to immunotherapy by intradermal skin test and antigen-specific IgE in canine atopy. *J. Vet. Med. Sci.*, 62: 983-988.
- Patel, M., D. Selinger, G.E. Mark, G.J. Hickey and G.F. Hollis, 1995. Sequence of the dog immunoglobulin  $\alpha$  and epsilon constant region genes. *Immunogenetics*, 41: 282-286.

- Prelaud, P., E. Guaguere, Z. Alhaidari, N. Faivre, D. Heripret and A. Gayerie, 1998. Reevaluation of diagnostic criteria of canine atopic dermatitis. *Rev. Med. Vet.*, 149: 1057-1064.
- Racine, B.P., E. Marti, A. Busato, R. Weilenmann, S. Lazary and M.E. Griot-Wenk, 1999. Influence of sex and age on serum total immunoglobulin E concentration in Beagles. *Am. J. Vet. Res.*, 60: 93-97.
- Roque, J.B., C.A. O'Leary, M. Kyaw-Tanner, M. Latter and K. Mason *et al.*, 2011. High allergen-specific serum immunoglobulin E levels in nonatopic West Highland white terriers. *Vet. Dermatol.*, 22: 257-266.
- Saevik, B.K., T.L. Ulstein and H.J.S. Larsen, 2003. Evaluation of a commercially available enzyme-linked immunosorbent assay for the detection of allergen-specific IgE antibodies in dogs. *Res. Vet. Sci.*, 74: 37-45.
- Scott, D.W., W.H. Miller and C.E. Griffin, 2001. Skin Immune System and Allergic Skin Disease. In: Muller and Kirk's Small Animal Dermatology, 6th Edn., Scott, D.W., W.H. Miller Jr. and C.E. Griffin (Eds.). W.B. Saunders Co., Philadelphia, PA, USA., pp: 574-601.
- Stedman, K., K. Lee, S. Hunter, B. Rivoire, C. McCall and D. Wassom, 2001. Measurement of canine IgE using the  $\alpha$  chain of the human high affinity IgE receptor. *Vet. Immunol. Immunopathol.*, 78: 349-355.
- Wilkie, J.S., J.A. Yager, P. Eyre and W.M. Parker, 1990. Morphometric analyses of the skin of dogs with atopic dermatitis and correlations with cutaneous and plasma histamine and total serum IgE. *Vet. Pathol.*, 27: 179-186.
- Willemse, A., 1986. Atopic skin disease: A review and re-consideration of diagnostic criteria. *J. Small Anim. Pract.*, 27: 771-778.
- Zunic, M., 1998. Comparison between IMMUNODOT tests and the intradermal skin test in atopic dogs. *Vet. Dermatol.*, 9: 201-205.