

Detection of allergen-specific antibody-secreting cells in dogs by ELISPOT

Michael P. Pelst^a, Elisa Maina^b, Sophie Vandenabeele^c, Myriam Hesta^d, Eric Cox^{a,*}^a Laboratory of Veterinary Immunology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium^b Servizi Dermatologici Veterinari. 2, via Don Pifferetti 2, Lovere BG, Italy^c Small Animal Department, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium^d Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, 9820 Merelbeke, Belgium

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ABSTRACT

Current laboratory tests are unable to distinguish healthy from allergic dogs. Unlike serum antibody responses, circulating antibody-secreting cells (ASC) are temporarily induced after each contact with the antigen. These ASC can be identified using ELISPOT and the observation of allergen-specific ASC might correlate with the causative allergens in dogs with an allergic dermatitis. In this study, blood was sampled from six privately-owned allergic dogs and six non-allergic laboratory beagles to determine the frequency of circulating allergen-specific ASC for common allergens. Blood IgE⁺, IgA⁺ and IgG⁺ cells were magnetically isolated to determine the number of allergen-specific ASC with ELISPOT for *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria alternata*, birch, timothy grass, wheat, cow's milk, bovine, chicken and lamb meat. For IgA and IgG, allergen-specific spots were observed, however for IgE, no spots were detected for any of the allergens. ELISPOT could not differentiate allergic from non-allergic dogs. When the responses to the different allergens were compared, more IgA ASC for *D. pteronyssinus* were observed compared to some of the other allergens which was statistically significant for the non-allergic dogs and approached significance in the allergic dogs. These findings indicate that ELISPOT can be used to identify circulating allergen-specific IgA- and IgG-secreting cells. The technique did however not detect allergen-specific IgE ASC and was unable to distinguish allergic from non-allergic dogs. Only a small number of studies have studied allergen-specific IgA in dogs. The finding that dogs have higher numbers of *D. pteronyssinus*-specific IgA ASC points out that apart from IgE and IgG, it might be interesting to include IgA measurements for certain allergens to analyse the complete spectrum of both the protective and pro-allergic antibody responses.

1. Introduction

Pruritic skin diseases are one of the most common conditions in small animal practice (Hill et al., 2006). It is estimated that approximately 10–15 % of all dogs suffer from atopic dermatitis (AD) (Hillier and Griffin, 2001; Depessemier et al., 2019). Data on the prevalence of adverse food reactions (AFR) in dogs is limited, according to a recent literature review approximately 1 % of the whole dog population is affected (Olivry and Mueller, 2017). Despite the high prevalence, no laboratory test has yet been successful in distinguishing healthy from allergic animals. The results of both intradermal tests and IgE serum tests show a lack of clinical correlation (Hillier and DeBoer, 2001; Pucheu-Haston et al., 2015). These tests are therefore mainly used to select the allergens for allergen-specific immunotherapy in order to treat dogs suffering from AD (Olivry et al., 2010). Patch tests (Bethlehem et al., 2012) and western blotting for IgE (Favrot et al.,

2017; Maina et al., 2018) can be used to select ingredients for an elimination diet but these tests and IgE serology (Foster et al., 2003) are of no value to directly diagnose canine AFR since they cannot identify the causative food allergens. Nonetheless, IgE serology for food allergens is still commonly used by general practitioners (Hardy et al., 2014). Likewise, testing for food allergen-specific salivary IgA and IgM cannot distinguish non-allergic dogs from dogs with clinical food allergy (Udraite Vovk et al., 2019). The lymphocyte proliferation test is currently the most promising laboratory test to identify the causative food allergens for canine AFR (Ishida et al., 2004).

Dogs can be sensitized to different environmental allergens, the most common being house dust mites, of which there are two major species: *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* (Sture et al., 1995; Saridomichelakis et al., 1999). Other important allergens are pollens from trees and grasses (Saridomichelakis et al., 1999; Roussel et al., 2013). With the exclusion of the Mediterranean

* Corresponding author.

E-mail address: eric.cox@ugent.be (E. Cox).<https://doi.org/10.1016/j.vetimm.2020.110101>

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region, birch pollen is the most common allergenic tree pollen in Europe (D'Amato et al., 2007). Both birch and timothy grass pollen are known to show high homology and cross-reactivity with pollen from trees of the *Fagales* order and grass extracts from the *Poaceae* family, respectively. Their allergen extracts can therefore be used to identify whether the individual is sensitized to these groups of tree or grass pollen (Lorenz et al., 2009). Additionally, sensitization to allergens of fungal origin, especially *Alternaria alternata*, was shown to be prevalent in Belgian dogs (Saridomichelakis et al., 1999). In dogs with AFR, the most common allergens are dairy products, wheat, bovine, chicken and lamb meat (Mueller et al., 2016).

When dogs are exposed to allergens, different allergen-specific antibody isotypes can appear in their blood. Immunoglobulin E was thought to play a primordial role in the pathogenesis of allergic diseases, being capable of priming mast cells for specific allergens. In dogs however, high titers of allergen-specific IgE or even a positive intradermal test are not necessarily associated with clinical hypersensitivity. Not only IgE antibodies are induced, also other immunoglobulin isotypes such as IgA and IgG antibodies are generated when the animal is exposed to allergens. Presence of such immunoglobulins in the dogs' serum would rather reflect allergen exposure than actual sensitization (Pucheu-Haston et al., 2015). Additionally, allergen-specific IgA and IgG antibodies could play a mechanistic role in the complex immunopathogenesis of the allergic disease in which they act as protective blocking antibodies, preventing the binding of the allergen to IgE (Jutel et al., 2003).

To study allergen-specific immunoglobulin responses, most often the concentration of serum antibodies is determined. On the other hand, it is also possible to analyse the antibody-secreting cells (ASC) circulating in the blood. Circulating ASC are known to be generated rapidly, approximately within 6–8 days after exposure to the antigen, followed by the disappearance of these cells in a period of days. Unlike serum antibody responses, antigen-specific ASC re-emerge and disappear in the blood after each re-exposure to the antigen. The detection of allergen-specific ASC would therefore reflect to which antigen the individual was exposed during the past week and thus, in the case of allergic diseases, could potentially be used to indicate which allergen caused the symptoms when the animal is presented to the veterinarian. One way to study ASC is the use of ELISPOT, a technique which quantifies ASC frequencies. This technique is sensitive since it has the capability to detect single ASC (Saletti et al., 2013). To improve sensitivity, the desired cell type can be enriched with the use of magnetic beads bound to antibodies (Lakew et al., 1997; Nguyen et al., 2015).

In this study, ELISPOT was used to assess whether blood-derived allergen-specific IgE, IgA and IgG ASC were restricted to or more numerous present in allergic dogs. Additionally, differences in isotype-specific responses to the different allergens were assayed.

2. Material and methods

2.1. Animals

Blood was sampled from healthy adult laboratory beagles with no history of an allergic disease and from adult dogs owned by clients of the Ghent University small animal clinic. These client-owned dogs had been previously diagnosed as suffering from allergic dermatitis other than allergic contact dermatitis or flea allergy and were presented to the veterinary dermatologist with recurrence of the pruritic symptoms. Diagnosis of allergy as cause of the symptoms was established based on the dog's history, clinical signs and the exclusion of parasitic and infectious skin diseases following standard procedures (Hensel et al., 2015). In these dogs no food trial was performed to distinguish AD from AFR. All dogs were maintained on appropriate flea prevention. All client-owned dogs had to show signs of pruritus at the time of blood sampling and a blood sample was collected once for each dog. Dogs were not included in the study when anti-inflammatory or anti-pruritic

medication was given within 2 weeks (oral glucocorticoids, topical glucocorticoids, antihistamines or oclacitinib) or 4 weeks (long-acting glucocorticoids or lokivetmab) prior to blood sampling. Dogs were also excluded when they had diagnosed tumours, kidney, liver, hormonal or auto-immune diseases. No general blood analysis was performed to identify dogs with subclinical disease. This study was approved by the local ethical committee of Ghent University, Belgium and the Flemish animal welfare service (EC2016/129-2017N02). Written informed consent was obtained from the owners of the pet dogs for blood sampling.

2.2. Blood sampling and peripheral blood mononuclear cell isolation

A volume of 8 mL blood was collected using a 21 G needle (Terumo, Leuven, Belgium) and immediately transferred to a lithium heparin Vacutainer tube (BD Biosciences, Franklin Lakes, NJ, USA). The samples were kept at room temperature (18–22 °C) (RT) until processing within 8 h after blood sampling. Blood was centrifuged at 230 g for 15 min at RT. Blood plasma was collected, aliquoted and frozen at –20 °C. Cells were resuspended 1:1 in PBS. Upon Ficoll density gradient centrifugation (900 g, 30 min, RT) (7.1 % Ficoll PM400 and 9% sodium diatrizoate hydrate (Merck, Burlington, MA, USA)) (De Bruin et al., 2005), the interphase containing the peripheral blood mononuclear cells (PBMC) was collected, diluted in an equal volume of Alsever's solution (pH of 6.1) (VWR, Radnor, PA, USA), centrifuged (300 g, 7 min, 18 °C) and the supernatant was removed. Red blood cells were lysed by adding 5 mL lysis buffer (140 mM NH₄Cl, 17 mM Tris (VWR), with a pH of 7.2) for 5 min at RT, the remaining cells were washed with 5 mL Alsever's solution (300 g, 7 min, RT) and finally resuspended in PBS at 10⁸ cells/mL.

2.3. Magnetic isolation of isotype-specific ASC for IgE, IgA and IgG

Ten million PBMC were incubated with 100 µg/mL goat anti-dog IgE FITC (Bio-Rad, Hercules, CA, USA) for 20 min on ice after which they were washed (400 g, 7 min, 4 °C) with EDTA-FCS buffer (PBS with 1 mM EDTA (VWR) and 1% heat-inactivated fetal calf serum (FCS) (Merck)). Anti-FITC microbeads (Miltenyi biotec, Bergisch Gladbach, Germany) ¼ (vol/vol) in EDTA-FCS buffer were added to the cells in a volume of 200 µL for 20 min on ice. After washing, cells were resuspended in 500 µL EDTA-FCS buffer and added onto an MS column (Miltenyi biotec), prewashed with 500 µL EDTA-FCS buffer and placed in an OctoMACS™ separator (Miltenyi biotec). The column flow-through (IgE[–] cell fraction) was collected. After removing the column from the separator, the retained IgE⁺ cells were eluted in a separate tube with 1 mL of EDTA-FCS buffer. Both cell suspensions were centrifuged (400 g, 7 min, 4 °C). The IgE[–] cells were resuspended in 100 µL of 10 µg/mL goat anti-dog IgA FITC (Bio-Rad) and incubated for 20 min on ice, whereafter the suspension was used to isolate the IgA⁺ cells and IgE-IgA- PBMC. The latter were incubated in 10 µg/mL FITC conjugated goat anti-canine IgG (H + L) (ThermoFisher scientific) to obtain the IgG⁺ cells and IgE-IgA-IgG- PBMC. After centrifugation and removal of the supernatant, the IgE⁺, IgA⁺ and IgG⁺ cells were resuspended in 1.2 mL of complete RPMI (RPMI 1640 supplemented with 10 % FCS, 2 mM L-glutamine, 1 % MEM non-essential amino acid solution, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 µg/mL streptomycin and 100 µg/mL kanamycin (ThermoFisher Scientific, Waltham, MA, USA)) and cell suspensions were kept on ice until added to a plate for ELISPOT.

2.4. Allergen-specific ELISPOT for IgE, IgA and IgG

Lyophilized protein extracts of *D. farinae*, *D. pteronyssinus*, *A. alternata*, birch (*Betula verrucosa*), timothy grass (*Phleum pratense*), bovine meat (*Bos taurus, carnem*), cow's milk (*Bos taurus, lac*), wheat (*Triticum aestivum*), chicken meat (*Gallus gallus, carnem*) and lamb meat (*Ovis*

Table 1
Summary on the number of animals, breed, sex and age of the dogs in the allergic and non-allergic group.

Allergy status	Number of animals	Breed	Sex	Age in years (min-max; mean $\pm$ SD)
Allergic dermatitis	6	Boxer, Doberman, German shepherd (2), Labrador retriever, mixed-breed dog	Male neutered (1), female intact (2), female neutered (3)	2–8; 4.8 $\pm$ 2.5
Non-allergic	6	Beagle	Male intact (2), male neutered (1), female neutered (2)	3–5; 4.3 $\pm$ 1

aries, carnem) (Greer laboratories, Lenoir, NC, USA) were dissolved in sterile PBS to a protein concentration of 5 mg/mL, aliquoted and stored at -20°C (Jeong et al., 2013). A Nunc Maxisorp 96-well plate (ThermoFisher Scientific) was coated with 50 $\mu\text{g/mL}$ of each of these allergen extracts in bicarbonate buffer with pH 9.4. Coating occurred for 8 h at RT, followed by blocking using bicarbonate buffer with 2% gelatin from cold water fish skin (blocking buffer) (Merck) for 2 h at RT. After washing (PBS + 0.05 % Tween®20 (Merck)), the IgE +, IgA + and IgG + cell suspensions were resuspended in complete RPMI and added undiluted in a volume of 50 $\mu\text{L/well}$ for 16 h at 37°C and 5% CO_2 . Cells were washed away and 0.5 $\mu\text{g/mL}$ of goat anti-dog IgE HRP (Novus Biologicals, Centennial, CO, USA), 80 ng/mL of goat anti-dog IgA HRP and 80 ng/mL goat anti-dog IgG (H + L) HRP (Bethyl, Montgomery, AL, USA) in blocking buffer was added for 2 h at RT to the wells where cells expressing the same isotype were seeded. After washing, 50 μL of TMB liquid substrate system for membranes (Merck) was added to each well for 10 min at RT. The fluid was carefully aspirated and the plate was scanned using an ImmunoSpot analyser (Cellular Technology Limited, Cleveland, USA). Simultaneously, the total number of ASC per isotype was determined using the same protocol on the same plate, but wells were coated with either 2 $\mu\text{g/mL}$ goat anti-dog IgG (H + L), 5 $\mu\text{g/mL}$ goat anti-dog IgA or 10 $\mu\text{g/mL}$ goat anti-dog IgE (Bethyl) and cell suspensions were added undiluted (IgE) or diluted 1/10–1/100 (IgA and IgG) to wells coated with antibodies of the corresponding isotype. Additionally, to determine the number of background spots, cell suspensions were added undiluted to uncoated wells. All conditions were assessed in duplicate for each dog. Spots were counted using the autocalculation function of the ImmunoSpot 4.0 software (Cellular Technology Limited). The quantity of allergen-specific spots was determined by subtracting the number of background spots from the number of spots in the wells coated with the protein extracts. For each antibody isotype, results were finally expressed as the number of allergen-specific ASC/1000 ASC.

2.5. Allergen-specific ELISA for IgE, IgA and IgG

The ELISA followed the procedure of the allergen-specific ELISPOT, blood plasma was diluted 1/50, 1/10 and 1/5 for detection of IgG, IgA and IgE, respectively for 2 h at RT. As substrate, 50 μL of 1 mg/mL ABTS (Merck) was added to the wells for 30 min at RT. Results are expressed as the optical density (OD) measured at 405 nm.

2.6. Statistical analysis

Statistical analysis was performed using R (R Core Team (2019)). Normality of the data was assessed using the Shapiro-Wilk's test and quantile-quantile plot. Since the data was not normally distributed, the Mann-Whitney *U* test (MWU) was used for comparisons between allergic and non-allergic dogs. The Wilcoxon signed-rank test was used to compare the responses to the different allergens. For all statistical tests, a *p*-value lower than 0.05 was considered significant. Results are reported as the mean $\pm$ standard deviation (SD). To generate the plots for the figures, the R ggplot2 package was used.

3. Results

3.1. Animals

Blood samples were collected from a total of 6 client-owned dogs with an allergic dermatitis and 6 laboratory beagles as healthy non-allergic control dogs. For each dog a blood sample was collected once. Sampling was performed throughout the year for the client-owned dogs while the blood samples of the laboratory beagles were collected in June. At the time of sampling, the client-owned dogs received either commercial maintenance diets, home-made or commercial hypoallergenic diets and often the dogs were given table scraps. All laboratory

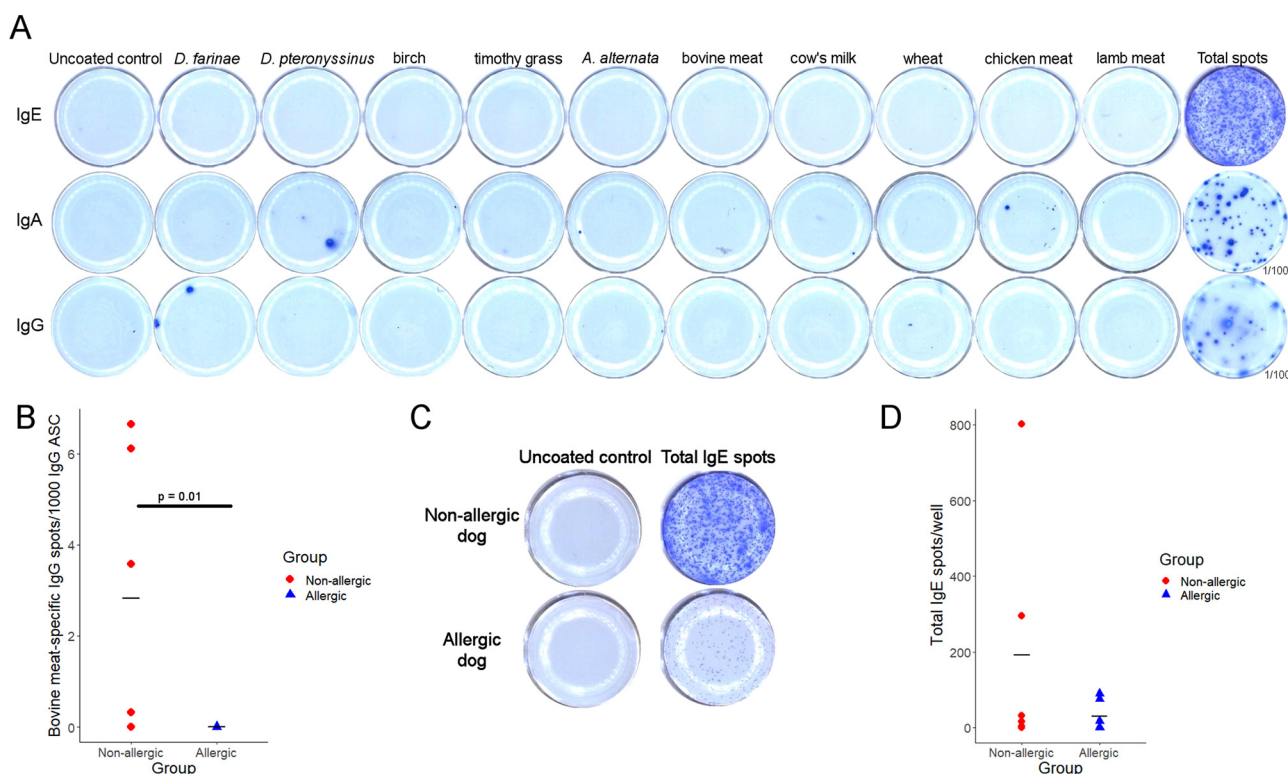


Fig. 1. Allergen-specific ELISPOT in non-allergic and allergic dogs. **A.** ELISPOT plate showing IgE, IgA and IgG ASC for the protein extracts of *D. farinae*, *D. pteronyssinus*, birch, timothy grass, *A. alternata*, bovine meat, cow's milk, wheat, chicken meat and lamb meat of a representative non-allergic dog. Total isotype-specific spots are shown in the last column, for total IgE in an undiluted cell suspension and for total IgA and IgG in a 1/100 diluted suspension. **B.** Scatterplot comparing the number of bovine meat-specific IgG spots/1000 IgG ASC in non-allergic dogs and allergic dogs with $P = 0.01$ (MWU). Horizontal bars indicate the mean. **C** ELISPOT wells showing the total IgE ASC with the corresponding uncoated negative control well in a non-allergic and allergic dog. **D.** Scatterplot showing the number of total IgE ASC in non-allergic and allergic dogs. Horizontal bars indicate the mean.

beagles were kept on a complete dry diet. A summary of the dogs' signalments is given in Table 1. A detailed overview on the age, sex, breed and diagnosed allergens of these dogs can be found in Supplementary Table A.

3.2. Allergen-specific IgE-, IgA- and IgG antibody-secreting cells (ASC) in allergic and non-allergic dogs

For IgE, no ASC were detected for any allergen in any dog, whereas for both IgA and IgG, ASC were observed for some allergens in most dogs (Fig. 1A). There were no significant differences in the number of allergen-specific ASC between non-allergic and allergic dogs, with the exception of the bovine meat-specific IgG for which the non-allergic dogs (2.8 ± 3.1 bovine meat-specific IgG spots/1000 IgG ASC) had a higher number of spots than allergic dogs (0 ± 0) ($P = 0.01$; MWU) (Fig. 1B). Although no allergen-specific IgE spots were detected, spots were generated for total IgE (Fig. 1C). There was no significant difference in the number of total isolated IgE ASC between non-allergic (191 ± 320) and allergic dogs (31 ± 41) ($P = 0.08$; MWU) (Fig. 1D).

3.3. Dogs show more IgA ASC for *D. pteronyssinus* compared to other allergens and more *D. Pteronyssinus*-specific IgA antibodies in blood plasma

When the results were analyzed from a different perspective and the isotype-specific response to the different allergens was compared, the number of *D. pteronyssinus*-specific IgA ASC were significantly higher compared to *D. farinae*, timothy grass, cow's milk, wheat and lamb meat in the non-allergic dogs. Additionally, approaching significance, allergic dogs had a higher number of *D. pteronyssinus*-specific IgA ASC compared to several of the other allergens tested (Fig. 2A). This difference could not be observed for the *D. pteronyssinus*-specific IgG ASC

(Fig. 2B). Higher IgA antibody concentrations were also observed for *D. pteronyssinus* in the blood plasma (Fig. 3).

4. Discussion

This study had several limitations. Due to the small number of dogs included, the nonsignificant results cannot be interpreted as being representative for the overall dog population. Additionally, allergic and non-allergic dogs were kept in different living environments, with the client-owned dogs living in common households and the laboratory beagles being held in a kennel. It can be assumed that dogs in kennels are exposed to different loads of allergens and have less contact with house dust mite due to limited exposure to mite-containing fabrics. Still, the laboratory beagles had ASC and antibody responses for *D. farinae* and *D. pteronyssinus* which were statistically indistinguishable from the dogs in the allergic group. Furthermore, the blood samples of the allergic dogs were collected during the entire year, while some of the allergens tested were seasonal allergens (*A. alternata*, timothy grass and birch) (Zureik et al., 2002; D'Amato et al., 2007). If all samples had been collected during the pollen or mold season, higher numbers of ASC might have been induced and a significant difference between these allergens and *D. pteronyssinus* for IgA ASC might not have been observed. Another limitation is that ASC appear in the peripheral circulation approximately one week after antigen exposure and, without any subsequent antigen contact, disappear within days (Saletti et al., 2013). In case the allergen only functioned as an initial trigger of the vicious cycle of skin inflammation (Marsella et al., 2012), it is possible that high numbers of allergen-specific ASC were generated but had disappeared by the time the dog was presented to the veterinary dermatologist. As a final limitation, FCS was used in the culture medium. FCS is of bovine origin and likely contains proteins similar to bovine meat,

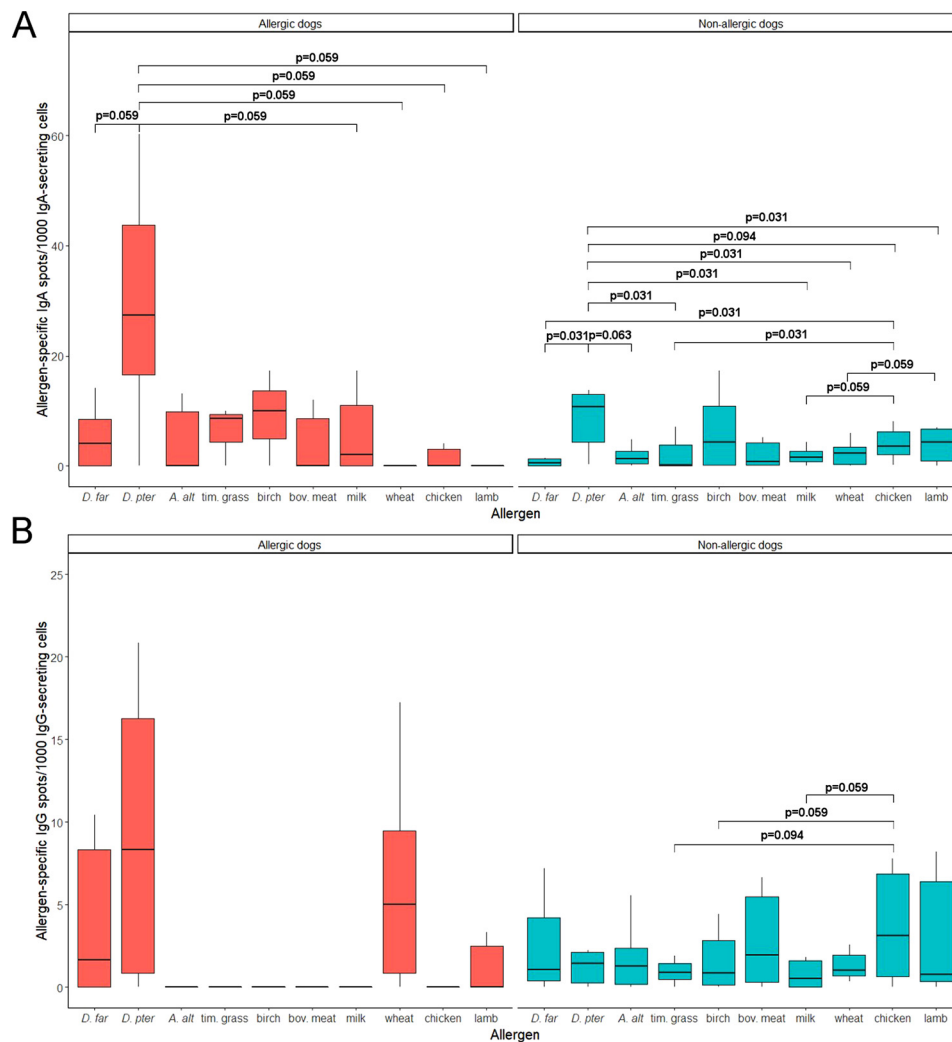


Fig. 2. Allergen-specific ELISPOT to detect IgA and IgG ASC for *D. farinae* (*D. far*), *D. pteronyssinus* (*D. pter*), *A. alternata* (*A. alt*), timothy grass (tim. grass), birch, bovine meat (bov. meat), cow's milk, wheat, chicken meat and lamb meat. **A.** Boxplots comparing the number of allergen-specific IgA spots/1000 IgA ASC for allergic and non-allergic dogs. P-values < 0.1 are given. **B.** Boxplots comparing the number of allergen-specific IgG spots/1000 IgG ASC for allergic and non-allergic dogs. P-values < 0.1 are given.

cow's milk or lamb meat. When ASCs were cultured in the presence of FCS, the bovine- or lamb-specific antibodies could have been captured by the proteins in the culture medium and therefore these antibodies would not have contributed to the generation of the spots, potentially leading to an underestimation of the number of spots for bovine meat, cow's milk or lamb meat.

To the authors' knowledge, this is the first study that used ELISPOT to determine the presence of allergen-specific ASC in dogs. One of the goals of the study was the detection of allergen-specific IgE-secreting B cells. However, in none of the dogs these cells were detected. In humans, circulating IgE + B cells are known to be scarce (Stone et al., 2010) and these cells only express limited surface IgE (Karnowski et al., 2006). It has even been shown that unlike the classical maturation of B cells, IgE plasma cells are mostly generated through class switching from IgG + memory B cells rather than IgE + memory cells in humans and mice (Erazo et al., 2007; Aranda and Curotto de Lafaille, 2019). This would indeed limit the number of IgE-secreting cells isolated with magnetic separation based on IgE expression, as was performed during this study. No allergen-specific IgE spots were detected during the assays, although for most dogs a limited amount of 'total' IgE-secreting cells were isolated. Apart from allergens, another known important inducer of IgE antibodies are parasites (Hill et al., 1995). It could therefore be that the isolated IgE-secreting cells produced antibodies for

antigens of parasitic origin, although no correlation was found between the frequency of deworming and the number of IgE ASC (results not shown). Not only parasites but also other infectious agents such as viruses and bacteria are capable of inducing pathogen-specific IgE antibodies and therefore possibly specific IgE ASC (Gershwin et al., 2000; Bexley et al., 2013).

When the number of IgA and IgG ASC was determined, a significantly higher IgA response for the house dust mite *D. pteronyssinus* was observed compared to several allergens for the non-allergic dogs. There is only limited knowledge on the role of immunoglobulin A in canine allergic diseases. Most studies focused on total IgA levels in the serum rather than allergen-specific IgA. One study showed a significantly decreased total IgA concentration when the animals suffered from ecto- or endoparasite infestation or from allergic dermatitis (Hill et al., 1995). Accordingly, in human children, serum and salivary *D. pteronyssinus*-specific IgA antibodies were significantly higher in healthy individuals compared to children with allergic rhinitis (Miranda et al., 2011). In our study, no significant difference in the number of allergen-specific IgA ASC was found between non-allergic and allergic dogs. Another study with dogs was unable to show a difference in IgA serum levels but could determine that atopic dogs had a significantly higher concentration of IgA in their skin compared to non-allergic control dogs (Mueller et al., 1997). IgA ASC of atopic dogs

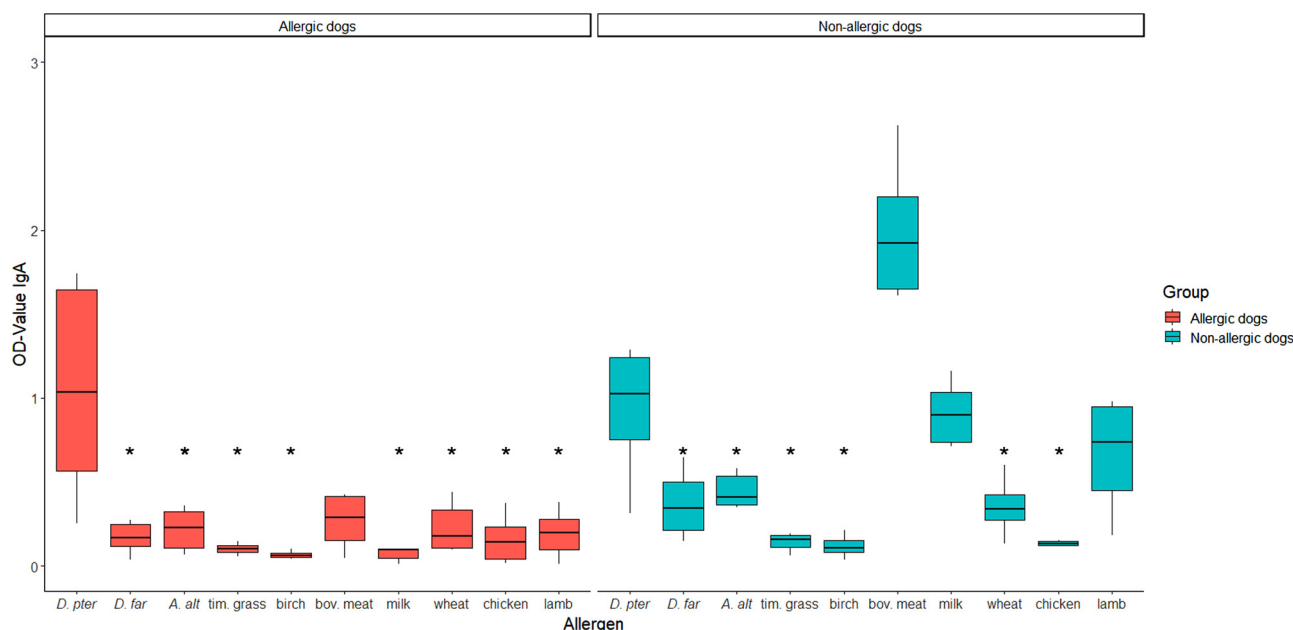


Fig. 3. Allergen-specific IgA ELISA for blood plasma showing the OD-value for *D. farinae* (*D. far*), *D. pteronyssinus* (*D. pter*), *A. alternata* (*A. alt*), timothy grass (*tim*), birch, bovine meat (*bov. meat*), cow's milk, wheat, chicken meat and lamb meat. Asterisks indicate a p-value < 0.05 compared to *D. pteronyssinus*.

might therefore be more prone to migrate to the skin where they locally produce IgA, resulting in higher skin concentrations of this immunoglobulin isotype.

A previous study has shown cross-reactivity between mite allergens of the *D. farinae* and *D. pteronyssinus* species (Saridomichelakis et al., 2008). However, in this study, the number of *D. pteronyssinus*-specific IgA spots and concentration of blood plasma IgA was significantly higher in the non-allergic dogs compared to *D. farinae* which suggests that dogs can mount a different type of immune response to the two mite species. A recent study using 2D-difference gel electrophoresis indeed showed that there was only a very limited overlap in proteins derived from *D. farinae* and *D. pteronyssinus* (Batard et al., 2016). The use of separate mite allergens for the diagnosis and treatment of canine allergies is therefore warranted.

5. Conclusion

This study evaluated whether circulating allergen-specific immunoglobulin-secreting cells were present in the blood of healthy and allergic dogs. In none of the dogs were allergen-specific IgE-secreting cells detected. Although IgA- and IgG-secreting allergen-specific ASC were observed, ELISPOT assays were unable to distinguish healthy from allergic dogs. Additionally, it was shown that non-allergic dogs had a significantly higher number of IgA-secreting cells for *D. pteronyssinus* compared to several of the other allergens tested. The complex immunopathogenesis of allergic diseases involves allergen-specific IgE-primed mast-cells, allergen-specific IgG antibodies with a potential blocking ability and both pro-allergic effector and anti-allergic regulatory T lymphocytes, each having its impact on promoting or inhibiting the generation of clinical signs in the animal. Apart from IgE and IgG antibodies, this study showed that dogs can also generate allergen-specific IgA ASC and high IgA antibody titers for a common environmental allergen. In order to analyse the complete spectrum of allergen-specific immune responses for canine allergies, the presence of allergen-specific IgA should therefore be considered.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.vetimm.2020.110101>.

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Supplementary Table A: Signalments of the 12 dogs of which the blood samples were used for both ELISPOT and ELISA measurements. An overview is given on the diagnosis of an allergic dermatitis, the breed, sex, age and diagnosed environmental allergens (IgE serology).

Allergy status	Breed	Sex	Age (years)	Diagnosed allergens
Allergic dermatitis	Boxer	Female neutered	8	Unknown
Allergic dermatitis	Dobermann	Female neutered	6	<i>Dermatophagoides farinae</i> , <i>Tyrophagus putrescentiae</i> , <i>Acarus siro</i>
Allergic dermatitis	German shepherd	Female intact	2	Unknown
Allergic dermatitis	German shepherd	Female neutered	3	<i>D. farinae</i> , <i>A. siro</i> , pollen of grasses
Allergic dermatitis	Labrador retriever	Male neutered	3	Unknown
Allergic dermatitis	Mixed-breed dog	Female intact	7	Unknown
Non-allergic	Beagle	Male intact	5	-
Non-allergic	Beagle	Male intact	5	-
Non-allergic	Beagle	Male neutered	3	-
Non-allergic	Beagle	Female intact	3	-
Non-allergic	Beagle	Female neutered	5	-

Non-allergic	Beagle	Female neutered	5	-
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