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Faecal IgA concentration is influenced by age in dogs

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Abstract

Data comparing age-related alterations in faecal IgA concentrations of dogs are not available in the literature. The present study aimed to compare the faecal concentrations of IgA in puppies, mature and senior dogs. A total of twenty-four beagle dogs were used, including eight puppies (5 months old, four females and four males), eight mature (4·6 years old, eight males) and eight senior dogs (10·6 years old, three males and five females). Fresh faecal samples were collected from each dog for three consecutive days and pooled by animal. After saline extraction, IgA content was measured by ELISA. Data were analysed by one-way ANOVA, and means were compared with Tukey's test ($P<0·05$). Results showed that puppies have lower faecal IgA concentrations than mature dogs ($P<0·05$); senior animals presented intermediary results. The reduced faecal IgA concentration in puppies is consistent with the reduced serum and salivary IgA concentrations reported previously, suggesting a reduced mucosal immunity in this age group. Although some studies have found an increased serum IgA concentration in older dogs, this may differ from the intestinal secretion of IgA, which appears to be lower in some senior animals (four of the eight dogs studied).

Key words: Ageing: Immunology: Mucosal immunity: Puppies

With the increase in the population of geriatric dogs, understanding how ageing influences immune parameters is important. Similar to human subjects and other animal models, the study of age-related alterations in the immune system aims to develop interventions to modulate and ameliorate these changes, which promotes the well-being and longevity of dogs. This is important because ageing may leave the individual more susceptible to infections and cancers and compromise the quality of their life and lifespan[1].

IgA is the most abundant class of antibody in mucous membranes, where it represents an essential factor in the protection against infectious agents, allergens and foreign proteins. In the intestine, it is mainly produced in Peyer's patches[2], and IgA secretion is used as an important indicator of the mucosal immunity status[3]. Among the factors that can influence IgA secretion, age, breed and diet were studied. When supplementing foods with specific substances, ileal or faecal IgA has been used to assess the dietary immunomodulatory effect[4,5].

Mucosal immunity development has been studied in some animal species. Studies in human subjects indicate that IgA synthesis does not occur during fetal life[6], and that significant salivary IgA levels are only found after 4–6 weeks of age, and these levels continue to increase up to 18 months of age[7]. Younger children have few IgA^+ cells in the intestine, and as they grow older, the number of these cells increase[8], which may explain the increase in IgA over time. Most of the studies about IgA production in dogs have used only animals over 2 years of age and measured serum and salivary IgA[9,10]. Only one study has evaluated IgA content in nasal secretions of puppies from birth to 6 weeks of age, and observed that IgA concentrations decrease markedly during the first 2 weeks after birth, after which it remained relatively constant[11].

Studies regarding immunosenescence in dogs have reported an age-related decrease in the proliferative response of blood mononuclear cells to mitogens, a decline in the number of peripheral blood lymphocytes, B-cells and T-cells, and a decreased ratio of CD4 + :CD8 + cells. Phenotypic alterations are accompanied by functional changes, such as a reduced ability to respond to stimulation by non-specific mitogens, relative change in the balance of T-helper 1 *v.* T-helper 1 CD4 + T-cell activity and a reduced delayed-type hypersensitivity response to mitogens[12]. These changes in peripheral blood lymphocytes also seem to occur within the intestinal lamina propria of ageing dogs with reduced T-cell

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numbers and a lower proliferative activity of intestinal cell populations[13]. However, this aspect has not been thoroughly studied in dogs. Alterations in IgA secretion related to age are not clear because aged mice and human subjects exhibit either increased or unchanged mucosal IgA concentrations[14]; in dogs, studies have described that both serum and salivary IgA may increase with age[9,10]. Considering this, we compared faecal IgA concentration in puppies, mature and geriatric dogs.

Materials and methods

A total of twenty-four beagle dogs were divided into three age groups of eight dogs each: puppies (5 months old; 5·2 (SEM 0·4) kg body weight, four females and four males, from three different litters); mature (4·6 (SEM 0·5) years old; 11·36 (SEM 0·55) kg body weight, eight males) and senior (10·6 (SEM 0·5) years old; 11·49 (SEM 0·95) kg body weight, five females and three males). All dogs were considered healthy after clinical and haematological examinations. Dogs were fed standard extruded kibble diets with similar compositions (24 % of crude protein; 14 % of acid-hydrolysed fat; 2 % of crude fibre and 8 % of ash on DM basis; composed by poultry meal, rice, maize, poultry fat, wheat bran, minerals and vitamins, and free of ingredients that may modulate gut immune status.

The study was conducted at the Laboratory of Research on Nutrition and Nutritional Diseases of Dogs and Cats, São Paulo State University, Jaboticabal, Brazil. All procedures were approved by the Ethics and Animal Welfare Committee of the Faculty of Agrarian and Veterinary Sciences, São Paulo State University according to the Brazilian animal protection law (protocol no. 021619/09).

For all dogs, fresh faecal samples (collected no later than 10 min after eliminated) were collected during three consecutive days and immediately frozen (−20°C). The samples were then thawed, pooled by dog and submitted for saline extraction, as described previously[15]. Extraction buffer (0·01 M-PBS, pH 7·4, 0·5 % Tween (Sigma-Aldrich, Poole, Dorest, UK) and 0·05 % sodium azide) was added to each tube at a ratio of 10 ml of buffer to 1 g (wet weight) of faeces. After homogenisation and centrifugation, the supernatant was transferred to a sterile eppendorf tube containing 20 μl of a protease inhibitor cocktail (Sigma-Aldrich). The samples were centrifuged at 15 000 ***g*** for 15 min at 5°C, and the supernatants were transferred to clean eppendorf tubes and stored at −20°C until analysis.

The quantification of IgA was performed using the ELISA kit for canine IgA determination (Bethyl Laboratories, Montgomery, TX, USA). Optical density was read at 450 nm with a Microplate Reader (MRX TC Plus, Dynex Technology, Chantilly, VA, USA). To calculate the IgA concentration, the optical density of the samples was compared with the optical density of a standard with a known concentration of IgA. The standard canine IgA sample was provided in the kit, and seven dilutions of the standard were made in order to develop a regression curve between optical density and IgA amount. All samples were tested in duplicate, and results are expressed as mean values.

Faecal DM was determined by sample drying at 105°C, and results are expressed as mg IgA per g of dry faeces. All analyses were carried out in duplicate with a CV of less than 5 %.

Statistical analysis

One-way ANOVA was used to compare variables across the three age classes. When a significant difference was identified by ANOVA ($P<0·05$), Tukey's test (*post hoc*) was used to identify differences among groups ($P<0·05$). Analysis was performed using the GraphPad Prism software (version 5.0; Graph-Pad Software, Inc., San Diego, CA, USA). All data were found to comply with ANOVA assumptions. Results are expressed as means with their standard errors.

Results

Puppies (0·32 (SEM 0·05) mg IgA per g of dry faeces) presented less faecal IgA ($P<0·05$) than mature dogs (2·34 (SEM 0·44) mg IgA per g of dry faeces). Senior dogs (1·45 (SEM 0·41) mg IgA per g of dry faeces) did not differ from either puppies or mature animals ($P>0·05$). A large deviation from the mean was observed for both mature and senior animals, but puppies had the most homogeneous distribution (Fig. 1).

Discussion

Studies of the effects of ageing on IgA secretion differ between species and authors. Results have shown that ageing can decrease, increase or have no effect on the number of mucosal IgA-producing cells[14,16]. The results of faecal IgA in puppies reported in the present study agree with the data reported for serum and salivary IgA concentrations[9,10], suggesting that as the dogs mature, an increase in IgA secretion was observed. However, for senior dogs, faecal IgA concentrations exhibited a slight reduction compared with mature dogs. While this result was not significant, it differed from the data observed for salivary IgA by HogenEsch *et al.*[10], which found higher salivary IgA concentrations in 12-year-old dogs

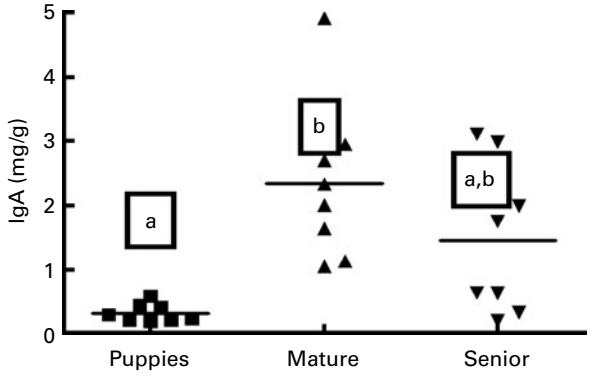

Fig. 1. Faecal IgA concentrations (mg/g of dry faeces) of puppies (*n* 8; ■), mature (*n* 8; ▲) and senior dogs (*n* 8; ▼). [a,b] Mean values with unlike letters were significantly different by Tukey's test ($P<0·05$).

compared with 3-year-old dogs. Interestingly, senior dogs could be separated in two groups: one group of four animals with >1·5 mg IgA per g of dry faeces and another group of four animals with <1 mg IgA per g of dry faeces.

Currently, the study of age-related changes in mucosal or secreted IgA concentrations is more applicable than performing extrapolations from the concentrations of serum IgA because serum IgA is not a good predictor of secretory IgA content[17]. Moreover, a higher serum IgA level observed in some elderly individuals may reflect a monomeric IgA increase that does not bind to the polymeric Ig receptor and is not transported to the mucosal surface as a secretory IgA[16].

Mucosal IgA measurements have been used for several reasons in veterinary and human medicine. This Ig is considered a marker of severity and progression of some gastrointestinal diseases in both dogs and human subjects[15]. In dogs, IgA deficiency has been correlated with chronic enteropathies; especially in German shepherd dogs, the lack of IgA would predispose to small-intestinal bacterial overgrowth[18,19]. Other methods can be used to assess mucosal immunity, e.g. immunohistochemistry to characterise leucocyte subsets, antibodies and cytokine-producing cells, or mucosal cell explants to study *ex vivo* cytokine production. However, these methods are more invasive, requiring gut biopsies, and were not used in the present study.

The reduced faecal IgA in puppies was not reported in the consulted literature. IgA secretion in the intestine is important because it actively binds to micro-organisms, enterotoxins and other antigens to prevent adherence and subsequent penetration into the gut wall[20]. Dogs are considered immunologically mature immediately after birth, but the immune response matures with age, and the humoral response, especially the IgA, seems to develop later[21]. Understanding changes in the immune system during growth and the nutritional interventions that may beneficially modulate their function could be important to the development of better diets for young animals.

For old dogs, the reported differences on faecal IgA concentration were not related to any identifiable condition, including age, sex, body weight or body condition, which were similar between the animals. Other studies in old dogs have also found large CV for IgA, 92[9] or 121 %[10], values even greater than those found in the present study (81 %). This probably refers to the fact that dogs, as humans, do not age consistently, and chronological age does not always match physiological age[22].

Ageing is associated with a general decline in the intestinal mucosal immune response[13], and our data suggest that some senior dogs also presented reduced faecal IgA concentrations. These alterations are linked to an increased morbidity and mortality due to infectious diseases in elderly individuals[16]. Thus, these animals might benefit from studies that enable a better comprehension of these changes and the nutritional interventions that could ameliorate them.

Conclusions

Puppies have reduced faecal IgA concentrations in comparison with mature dogs. Senior dogs presented intermediary faecal IgA concentrations, but some animals presented very low faecal IgA levels.

Acknowledgements

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