Effect of age, breed size and enteropathogen infection on fecal immunoglobulin A concentrations in weaning puppies

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Digestive health is a main concern for growth, morbidity and mortality in weaning puppies. Fecal immunoglobulin A (IgA) has been suggested as a useful noninvasive biomarker for mucosal immunity. The purpose of this study was to evaluate the effect of infection with enteropathogens on fecal IgA concentrations in puppies and that of physiological factors such as age and breed size.

282 puppies from 33 breeding kennels were included in the study. Puppies were between 5 and 14 weeks of age (mean±Standard Deviation (SD): 7.8±1.5 weeks). Depending on the mean adult body weight of their respective breed, the puppies were divided into small (if mean adult body weight < 25 kg) or large (>25 kg) breed puppies. For each puppy, fecal consistency was evaluated using a 13-point scale and feces were collected for the evaluation of presence of fecal enteropathogens and fecal IgA concentrations. The presence of enteropathogens in fecal samples was evaluated by qPCR for canine parvovirus type 2 (CPV2), qRT-PCR for canine coronavirus (CCV), coproantigen quantification for Giardia (ProSpecT-Giardia, Remel), and McMaster flotation technique for other parasite eggs and oocysts. Fecal IgA concentrations were measured by an ELISA test. Statistical analyses were performed using SAS software. A linear mixed model (proc MIXED) with fecal IgA

concentration as outcome was used to determine the following effects: enteropathogen infection, breed size, age, and fecal score. The respective influence of litter and breeding kennel as random effects was also determined. Data is presented as mean ± SD.

Small breed dogs represented 27.3 % (77/282) of the total number of dogs included. At least one enteropathogen was identified in 76.2 % of puppies (214/281). Fecal IgA concentration was significantly influenced by fecal enteropathogens ($p=0.037$). Puppies infected with at least one enteropathogen had significantly lower fecal IgA concentrations than puppies without any enteropathogens (5.0 ± 4.4 µg/g vs. 6.9 ± 5.5 µg/g). Breed ($p=0.029$), but not age ($p=0.082$), influenced IgA concentration. Small breed puppies had significantly higher fecal IgA concentrations than large breed puppies (6.8 ± 4.8 µg/g vs. 5.0 ± 4.7 µg/g). No significant relationship between fecal IgA concentration and feces quality was evidenced ($p=0.165$).

This study suggests that fecal IgA concentration is a promising marker for subclinical infection by at least one enteropathogen and confirms that digestive physiology varies with the breed size. A link between lower digestive immunity and higher susceptibility to enteropathogen infection needs further investigation.

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Introduction

Digestive health is a main concern for growth, morbidity and mortality in weaning puppies. Secretory immunoglobulin A (IgA) is the predominant immunoglobulin class present in secretions, protecting mucosal surfaces from infectious agents. Fecal IgA has been suggested as a noninvasive biomarker for mucosal immunity. **The purpose of this study was to evaluate the effect of enteropathogens infection on fecal IgA concentrations in puppies and that of physiological factors such as age and breed size.**

Materials and methods

260 puppies from **32 breeding kennels** were included in the study. Only puppies with a normal clinical examination were included (puppies with clinical signs of prostration, dehydration and/or anorexia were excluded from this study). Depending on the mean adult body weight of their respective breed, puppies were divided into **small** (mean adult body weight < 25 kg) or **large** (>25 kg) breed puppies. For each puppy, fecal consistency was evaluated using a 13-point scale adapted for fecal scoring in puppies [1]. Feces were collected to evaluate presence of fecal enteropathogens and to assay fecal IgA concentrations. Detection of enteropathogens was perfomed by qPCR for canine parvovirus type 2 (**CPV2**), qRT-PCR for canine coronavirus (**CCV**), coproantigen quantification for ***Giardia duodenalis*** (ProSpecT-Giardia, Remel), and McMaster flotation technique for **conventional coproscopy**. **Fecal IgA concentrations** were measured by an ELISA test as previously described [2].

Statistical analysis was performed using R software (R package version 1.1-7 Vienna, Austria). A linear mixed model (lme4) was used to evaluate effect of enteropathogens infection and breed size, as fixed effects, on IgA concentrations. Age, fecal score and litter nested in breeding kennel were modeled as random effects. A logarithmic transformation of this outcome was required to obtain a normal distribution of the residuals. Data are presented as median and range.

Results

Prevalence of enteropathogens

• Large breed dogs representing 73.8% (192/260) of the total number of dogs included. Puppies were between 5 and 14 weeks of age (median=8 weeks).

• Six different viruses and parasites were identified. 75% (195/260) of puppies were infected by at least one enteropathogen:

- 37.7% were infected by *Giardia duodenalis*,
- 30.8% by *Cystoisospora ohioensis*-complex,
- 21.9% by *Toxocara canis*,
- 20 % by CCV,
- 13.8% by CPV2,
- 10.4% by *Cystoisospora canis.*

Factors influencing fecal IgA concentration

• Fecal IgA concentration was significantly associated with breed size and *G. intestinalis* infection (Figure 1).

• Large breed puppies presented a significantly lower fecal IgA concentration than small breed puppies (4 [0.1-27.2] mg/g vs 5.8 [0.1-19.2] mg/g; P=0.034; Figure 2).

• Puppies infected by *G. duodenalis* presented a significantly lower fecal IgA concentration than puppies not infected by this parasite (4.3 [0.1-22.7] mg/g vs 5 [0.1-27.2] mg/g; P=0.047; Figure 3).

Figure 1: Effect of physiological and pathological factors on fecal IgA concentration

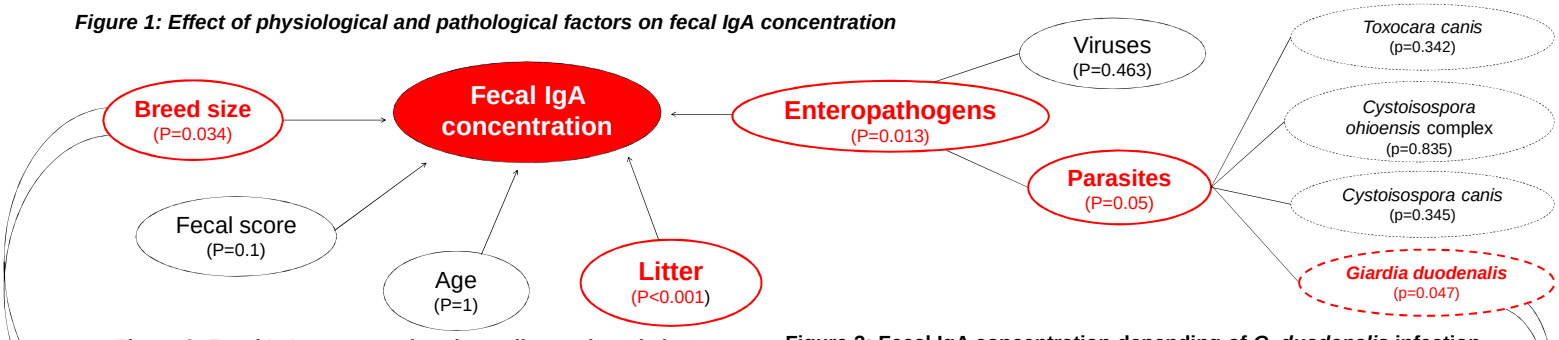


Figure 2: Fecal IgA concentration depending on breed size

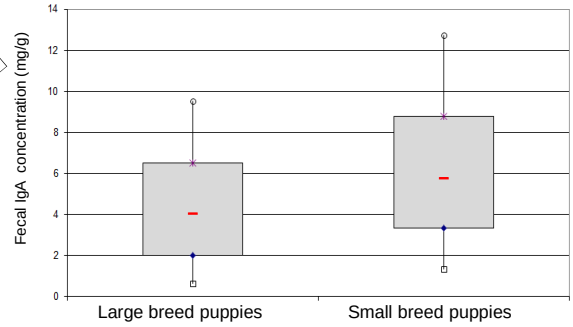
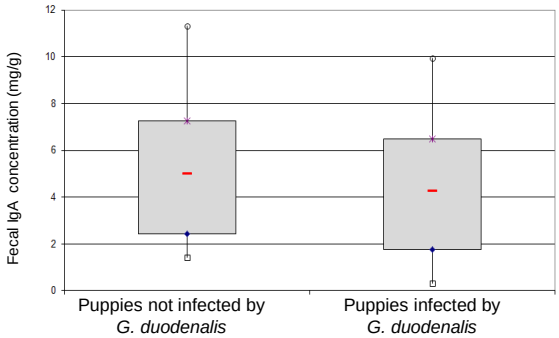


Figure 3: Fecal IgA concentration depending of *G. duodenalis* infection



Discussion

Effect of breed size on fecal IgA concentration: Significantly higher fecal IgA concentrations were observed in small breed size puppies compared to large breed size puppies. An effect of dog breed was also demonstrated in adult dogs with higher fecal IgA concentrations in small sized breeds [3].

Effect of parasites on fecal IgA concentration: The lower concentrations of fecal IgA in puppies infected by *G. duodenalis* could underline an active downregulation of the intestinal immunity by this parasite or an impaired gastrointestinal immunity promoting *G. duodenalis* infection as already demonstrated in mice [4].

Effect of viruses on fecal IgA concentration: A concentration of CPV2 coproantibodies was already described as influencing prognosis for parvovirosis infection (dogs with high levels of CPV2 coproantibodies having a favorable clinical prognosis). However, no significant effect of CPV2 on fecal IgA concentration was observed in our study. This could be due to the fact that only puppies without severe clinical signs were included in our study.

Conclusion

This study underlines that fecal IgA concentrations vary markedly depending of physiologic and pathologic factors like breed size and parasite infections. These factors need to be considered when fecal IgA is used for evaluation of intestinal immunity. Further investigation is necessary in order to understand the relationship between fecal IgA and intestinal pathogens.

[1] Grellet A. et al. Prev. Vet. Med. 2012 106 (3-4): 315-323; **[2]** Tress U. et al. Am. J. Vet. Res. 67, 1756-1759; **[3]** Peters IR. et al. Clin Vaccine Immunol. 2004,11 (5): 841-848; **[4]** Langford TD. et al. Infect. Immun. 2002; 70 (1): 11-18