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Risk factors of weaning diarrhea in puppies housed in breeding kennels

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ABSTRACT

Diarrhea represents one of the most frequent disorders in dogs. In puppies, degradation of feces quality is associated with a reduced daily weight gain and an increased risk of death. Prevention of diarrhea in puppies requires a global approach encompassing enteropathogens, environment and management practices especially when housed in groups. The purpose of this study was to determine prevalence of enteropathogens in puppies in breeding kennels and to identify risk factors of diarrhea. Two hundred and sixty six puppies (between 5 and 14 weeks of age) from 29 French breeding kennels were included. For each kennel, data about environment, management of the kennel and puppies' characteristics (age, sex and breed) were collected. For each puppy, fecal consistency and fecal excretion of enteropathogens (viruses and parasites) was evaluated. At least one enteropathogen was identified in 77.1% of puppies and 24.8% of puppies presented abnormal feces. The main risk factor of weaning diarrhea was fecal excretion of canine parvovirus type 2 (odds ratio = 5; confidence interval 95%: 1.7–14.7). A targeted sanitary and medical prophylaxis against canine parvovirus type 2 should be implemented to decrease risk of weaning diarrhea.

1. Introduction

Gastrointestinal and hepatic diseases in dogs are the third most frequent problem reported by owners in United States and Australia (Freeman et al., 2006). Diarrhea represents one of the most frequent disorders in dogs examined at private veterinary practice, with a prevalence of 2.2% (Lund et al., 1999), young dogs under 6 months of age being at a higher risk of diarrhea than adult dogs (Tupler et al., 2012). In puppies, degradation of feces quality is associated with a reduced daily weight gain and an increased risk of death (Grellet et al., 2012).

A great variety of parasites and viruses are described to be enteropathogens during the weaning period in puppies. *Giardia duodenalis*, *Cryptosporidium parvum*, *Toxocara canis*, *Cystoisospora ohioensis* complex, *Cystoisospora canis*, canine parvovirus type 2 (CPV2) and canine coronavirus (CCV) are the most prevalent (Hackett and Lappin, 2003). However, as

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in other species, diarrhea is multifactorial, involving factors intrinsic to the dog (breed size and age), nutritional factors (diet change without transition, food type and quality), together with lifestyle and environmental stressors (Weber et al., 2002, 2003; Sokolow et al., 2005; Hernot et al., 2006; Stavisky et al., 2011). Most studies on risk factors of diarrhea in young dogs focused on one single pathogen or a group of pathogens without taking into account environmental stressors (Finlaison, 1995; Buehl et al., 2006; Grellet et al., 2012; Tupler et al., 2012). Moreover most of the studies considering multiple enteropathogens infections were performed in shelters, in a context far different from that in breeding kennels (Sokolow et al., 2005; Tupler et al., 2012). The purpose of this epidemiological study was to determine prevalence of enteropathogens in puppies in breeding kennels and to perform a risk factors analysis for diarrhea during the weaning period including enteropathogens, environment and management procedures.

2. Materials and methods

2.1. Animals and breeding kennels

A total of 266 puppies (60 litters) from 29 French breeding kennels were included in this study between May and September 2009 (mean of 9 puppies included per kennel; range: 2–18). Puppies were between 5 and 14 weeks of age (mean: 7.8 weeks of age) (Fig. 1). These breeding kennels were randomly selected from a data base of breeders registered at Alfort Veterinary School for training programs. Only puppies with a normal clinical examination were included (puppies with clinical signs of prostration, dehydration and/or anorexia were excluded of this study). For each kennel, data concerning environmental factors (number of puppies sold per year, and litter size for each puppies included), management of the kennel and puppies (number of meals distributed per day, access to outdoor, vaccination) and puppies' characteristics (age, breed, sex), were collected. Puppies vaccinated within the preceding 10 days before the visit were not included.

Depending on the mean adult body weight of their respective breed, puppies were divided in two groups (small if mean adult body weight < 25 kg; large otherwise). Small breed dogs represented 25.6% (68/266) of the total number of dogs included. Based on the mean number of puppies sold per year (calculated over the last two years and considered as the size of the kennel), kennels were also separated into "small" (i.e. less than 30 puppies sold per year) and large kennels (i.e. more than 30 puppies sold per year). Puppies housed in breeding kennels producing 30 puppies or more per year represented 51.1% (136/266) of the total number dogs included. Puppies were divided into two groups according to the number of meal per day: puppies receiving less than 4 meals per day and puppies receiving 4 meals per days or more.

2.2. Evaluation of feces consistency

For each puppy, fecal consistency was evaluated by a single operator using a 13-point scale, based on the texture and shape of the feces (from liquid to hard and dry) (Grellet et al., 2012). Based on growth rate, thresholds for abnormal feces were previously validated and appeared to vary with breed stature and age (Grellet et al., 2012). Briefly, feces with a score ≤ 5 was classified as abnormal for large breed puppies whatever the age, for small breed puppies, fecal scores ≤ 6 and ≤ 7 were classified as abnormal for 4–5 weeks old puppies and for older puppies between 6 and 8 weeks old, respectively.

After collection, stools were separated in three samples, one being stored at +4 °C for coproscopy and other frozen (−20 °C) for *Giardia intestinalis* and *Cryptosporidium parvum* copro-antigens quantification.

A rectal swab was performed for each puppy immediately after stool collection for detection of canine parvovirus type 2 (CPV2) and canine coronavirus (CCV). The swabs were stored at −20 °C until DNA extraction.

2.3. Intestinal parasites

By the standard McMaster flotation technique using saturated magnesium sulphate solution (density: 1.28 g/ml) (Bauer et al., 2010), all eggs and oocysts were identified according to their morphological characteristics under light microscopy by a single operator (Levine and Ivens, 1965; Baek et al., 1993).

Copro-antigens of *G. intestinalis* and *C. parvum* were quantified on 100 mg of feces using respectively the ProSpecT-Giardia and the ProSpecT-Cryptosporidium Microplate Assay kit (Remel, France) (Decock et al., 2003; Mekaru et al., 2007; Rimhanen-Finne et al., 2007). An optical density value > 0.05 was considered positive according to the manufacturer's instructions.

2.4. Coronavirus and parvovirus fecal excretions

CPV2 and CCV detection were performed by qPCR and qRT-PCR respectively as already described (Grellet et al., 2012). Results from duplicate analyses (mean of two results) were expressed semi-quantitatively as viral load levels. Puppies were defined as excreting CPV2 and CCV for high viral loads over $10^{10.3}$ copies and $10^{9.3}$ copies respectively (Grellet et al., 2012).

2.5. Data management and statistical analysis

Statistical analyses were performed with the SAS version 9.3 software (SAS Institute Inc., Cary, NC, USA).

2.5.1. Statistical analysis for prevalence of enteropathogens

Number of puppies with fecal positive and negative test results for each enteropathogen was tabled by different factors under study like age of puppies, size of the kennel, breed size, and litter size. Univariate analyses of the putative risk factors for each enteropathogen infection were performed. The significance of the univariate associations was determined using the χ^2-tests. A P value < 0.05 was considered statistically significant.