# Risk factors of weaning diarrhea in puppies housed in breeding kennels

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## Introduction

Diarrhea represents one of the most frequent disorders in dogs examined at private veterinary practice, with a prevalence of 2.2%. Young dogs under 6 months of age present a higher risk than adult dogs. In puppies, degradation of feces quality is associated with a reduced daily weight gain and an increased mortality rate (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. Most studies on risk factors of diarrhea in young dogs focused on one single pathogen or a limited group of pathogens without taking into account environmental factors. Moreover, most of the studies considering multiple enteropathogens infections were performed in shelters, in a context far different from that in breeding kennels.

**The purpose of this epidemiological study was to determine the prevalence of enteropathogens in puppies in breeding kennels and to identify risk factors for diarrhea during the weaning period including enteropathogens, environment and management procedures.**

## Materials and methods

### Animals and breeding kennels.

A total of 266 puppies (60 litters) from 29 French breeding kennels were included in this study (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). These breeding kennels were randomly selected from a data base of breeders registered at Alfort Veterinary School for training programs. For each kennel, data concerning environmental factors, management of the kennel, and puppies' characteristics were collected (table 1).

| Variables | Categories | |
|---|---|---|
| Dog size | Small (adult body weight <25 kg) 26 % puppies | Large (adult body weight > 25 kg) 74 % puppies |
| Size of the kennel | Small (<30 puppies sold/year) 49 % puppies | Large (>30 puppies sold/year) 51 % puppies |
| Number of meals / day | < 4 meals / day 65 % puppies | ≥ 4 meals / day 35 % puppies |

*Table 1: Categories of the different variables evaluated*

### Evaluation of feces consistency.

For each puppy, fecal consistency was evaluated using a 13-point scale, based on the texture and shape of the feces (from liquid to hard and dry) (Figure 1). As previously described, feces were classified as abnormal or not (Grellet et al, 2012).

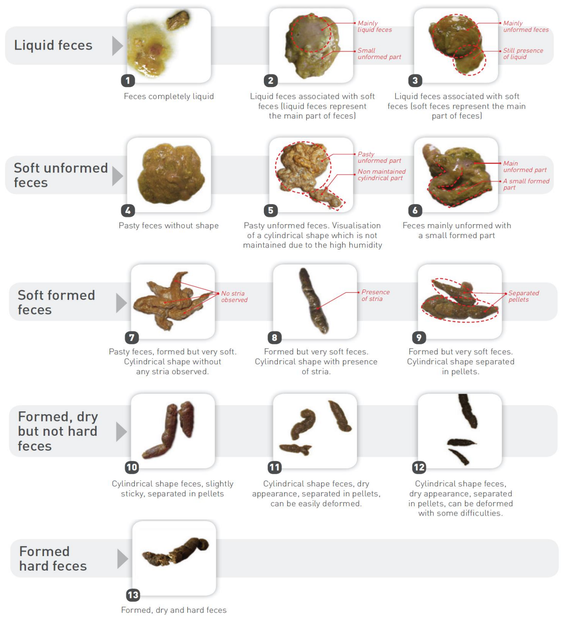


*Figure 1:13-point scale evaluating fecal consistency (Grellet et al, Prev Vet Med. 2012;106(3-4):315-23)*

### Fecal enteropathogens excretion.

By the standard McMaster flotation technique using saturated magnesium sulphate solution, all eggs and oocysts were identified according to their morphological characteristics under light microscopy. Copro-antigens of *G. intestinalis* and *C. parvum* were quantified using respectively the ProSpecT-Giardia and the ProSpecT- Cryptosporidium Microplate Assay kit (Remel, France). Canine Parvovirus 2 (CPV2) and Canine Coronavirus (CCV) detection were performed by qPCR and qRT-PCR respectively as already described (Grellet et al, 2012).

### Data management and statistical analysis.

Statistical analyses were performed with the SAS version 9.3 software (SAS Institute Inc., Cary, NC, USA). Correlation matrix of the different variables was determined with Kendall's Tau-b measure of correlation coefficient (Proc CORR). Highly correlated variables were defined as predictors in a partial least squares regression (Proc PLS) with fecal consistency as the response variable. The Variable Importance for Projection (VIP) was used to assess the contribution of each predictor to the model. Only predictors with a VIP value over 0.8 were included as a fixed effect in a generalized linear mixed model (proc GLIMMIX) with fecal consistency as a binary outcome (normal / abnormal) and Litter variable nested within breeding kennel as a random term.

## Results

**Prevalence of enteropathogens.** Seven different enteropathogens (2 viruses and 5 parasites) were identified within all tested samples (Table 2). 77.1% (205/266) of the puppies were infected by at least one enteropathogen with 29.3% of them excreting 3 pathogens or more (Figure 2). A significant effect of age and kennel size on the prevalence of enteropathogens was observed, except for *C. parvum*, with a significantly higher prevalence in large breeding kennels (Table 2).

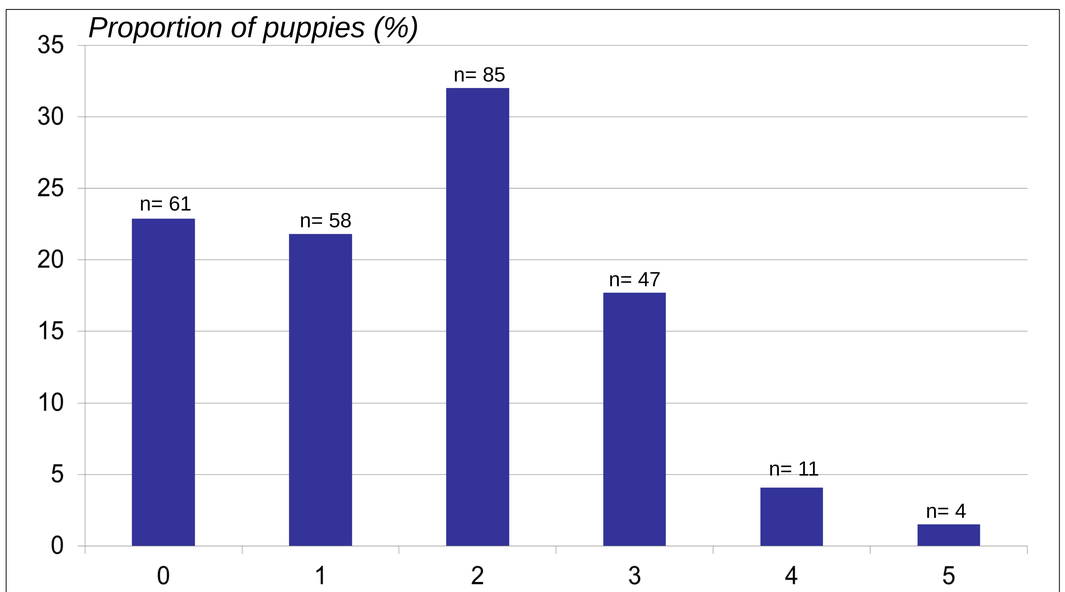


*Figure 2: Total number of enteropathogens identified per puppy (n=266 puppies)*

| Pathogens | Total prevalence | Age of puppies | | | | Size of the kennel | | |
|---|---|---|---|---|---|---|---|---|
| | | 5-6 weeks | 7-8 weeks | 9-14 weeks | Global P value | < 30 | ≥ 30 | P value |
| **CPV2** | 14.7 | 23.3 [a] | 16.7 [a] | 7.1 [b] | **0.032** | 0 | 28.7 | **<0.001** |
| **CCV** | 20.3 | 7 [b] | 13.8 [b] | 37.6 [a] | **<0.001** | 0 | 39.7 | **<0.001** |
| ***T. canis*** | 22.2 | 44.2 [a] | 22.5 [b] | 10.6 [c] | **<0.001** | 29.2 | 15.4 | **0.007** |
| ***C. ohioensis* complex** | 25.6 | 30.2 [a] | 31.9 [a] | 12.9 [b] | **0.005** | 23.8 | 27.2 | **0.002** |
| ***C. canis*** | 13.2 | 41.9 [a] | 6.8 [b] | 10.6 [b] | **<0.001** | 1.5 | 24.3 | **<0.001** |
| ***G. duodenalis*** | 41 | 32.6 [b] | 30.4 [b] | 62.4 [a] | **<0.001** | 17.7 | 63.2 | **<0.001** |
| ***C. parvum*** | 25.9 | 37.2 | 22.5 | 25.9 | 0.156 | 20.8 | 30.9 | 0.06 |

*Table 2: Prevalence of enteropathogens depending of puppies' characteristics and environmental factors.*

**Risk factors of abnormal feces** Sixty six out of 266 feces evaluated (24.8%) were classified as abnormal. In the initial partial least squares regression, CPV2, *C. canis*, *G. intestinalis* and the number of meal per day presented a VIP over 0.8. These four factors were included in a new partial least squares regression.CPV2 and number of meals per day were two factors keeping a significant impact on the incidence of abnormal feces (VIP = 1.7 and VIP = 1.0 respectively).

In the final model, **only fecal excretion of CPV2 increased risk of weaning diarrhea** (P = 0.003, odds ratio = 5; confidence interval 95%: 1.7–14.7). 61.5% (24/39) of puppies infected by CPV2 presented abnormal feces compared to 15.2% (42/277) of puppies not infected by CPV2. A significant effect of the litter (P < 0.001) and no significant effect of the breeding kennel level (P = 0.101) were observed.

## Discussion

- The **high prevalence of all enteropathogens tested** can be explained by the age of animals ("immunity gap" during the weaning period), their lifestyle (high exposition in dogs living in breeding kennels) and methods used for their detection (high sensitivity of PCR and ELISA tests).
- Although prevalence of enteropathogens is high, **only CPV2 was associated with diarrhea in puppies** without any systemic sign. This mild clinical signs could be explained by either an efficient systemic immunity (high level of maternally derived antibodies) or by an efficient local intestinal immunity (high fecal IgA concentration).

## Conclusion

**Based on this study, CPV2 infection was the major risk factor of weaning diarrhea.**

**Some central strategies can be suggested like a targeted sanitary and medical prophylaxis against CPV2, particularly in large breeding kennels.**