

Canine Parvovirus (CPV) and Intestinal Parasites: Laboratorial Diagnosis and Clinical Signs From Puppies With Gastroenteritis

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ABSTRACT

Canine parvovirus (CPV) infection is still spread among puppies in the State of Rio de Janeiro, Brazil despite the use of vaccination. The evolution of CPV enteritis depends on the host's age, general condition, and on preexisting or concurrent parasitic infections. Fifty-two fecal samples collected in puppies up to 6 months old with gastroenteritis from November 2002 to July 2004 were tested for CPV infection and intestinal parasites. Eighteen samples (34.6%) were positive for CPV and 12 (23%) were positive for intestinal parasites. The clinical signs of more severe enteritis (vomiting, anorexia, lethargy, and hemorrhagic fluid diarrhea) was observed in 10 of the 18 CPV-positive puppies and in 3 of the 12 puppies with parasitic infection. The concurrent CPV and detectable parasitic infection was found in 2 samples. Only 1 puppy with concurrent CPV and *Ancylostoma* spp presented signs of severe disease. For the remaining puppies, the clinical signs varied from soft diarrhea to

acute dysentery. These results show that in cases of hemorrhagic or nonhemorrhagic diarrhea with or without vomiting, lethargy, or anorexia, laboratorial diagnosis is essential to confirm CPV or parasitic infection.

INTRODUCTION

Management and control of canine enteritis is difficult. Besides viral agents, there are other important etiologic causes of enteritis such as bacteria and parasites.¹ In Brazil, since the early 1980s, parvoviral enteritis is recognized as one of the most common causes of infectious diarrhea in puppies up to 6 months of age.²⁻⁴ As canine parvovirus (CPV) replicates only in dividing cells, any agent (parasitic, bacterial or virus) that causes destruction of the villous, stimulating mitosis in intestinal tissues, may facilitate CPV replication and, as result, cause a more severe disease.⁵

It has been recognized that some intestinal parasites as *Toxocara canis*, *Ancylostoma caninum*, *Giardia duodenalis*, and coccidia are considered clinically important cause of gastrointestinal disease in dogs, especially puppies.⁶⁻⁹ There are few case

reports documenting *Cryptosporidium parvum* infection in dogs, probably because infection is subclinical in most individuals, but concurrent infections with virus have already been described causing severe intestinal disease.^{10,11}

The purpose of this study up was to perform the laboratorial diagnosis of CPV infection and intestinal parasites in puppies with gastroenteritis up to 6 months old and search for concomitant infection that may increase the severity of CPV illness.

MATERIALS AND METHODS

A total of 52 fecal samples, collected from November 2002 to July 2004 from puppies up to 6 months old with diarrhea were obtained from private and public animal hospitals located in Rio de Janeiro City. With consent of the owners of the animals, the stool samples were collected after spontaneous fecal discharge. Information regarding sex, age, days of disease, clinical findings, and CPV vaccination history was drawn from the records.

Part of the fecal samples were stored at -20° C for CPV diagnosis by hemagglutination/hemagglutination-inhibition (HA/HI) tests with swine erythrocytes.^{2,12} All HA/HI-positive samples from vaccinated puppies were submitted to PCR with differential primers in an attempt of distinguishing wild from modified-live vaccine virus.²

The remaining sample was kept in Railliet-Henry solution until processing for parasitological diagnosis: fecal flotation,¹³ formalin-ether sedimentation,¹⁴ and formalin-ethylacetate sedimentation.¹⁵ *Cryptosporidium* was screened employing stool smears submitted to concentration by the Ritchie modified technique¹⁴ and were stained by modified Safranin.¹⁶

RESULTS

Fecal samples from 31 male and 20 female puppies showing signs of gastroenteritis were tested. Eighteen samples (34.6%) were CPV positive and 12 (23%) were intestinal parasites positive. For 1 puppy, negative for CPV and parasitic infection, sex data was not recorded.

Single parasite infection was found in 9 samples: *Cystoisospora canis* (1), *Cystoisospora ohioensis* (1), *G duodenalis* (3), *T canis* (1), *Ancylostoma* spp (3), and multiple parasitism in 3 samples: *C canis* + *C ohioensis* (1), *Ancylostoma* spp + *T canis* (1), and *C ohioensis* + *Ancylostoma* spp + *T canis* (1). Concomitant CPV and parasitic infection was detected in only 2 samples: CPV + *C canis* + *C ohioensis* (1) and CPV + *Ancylostoma* spp (1). No cyst of *Cryptosporidium* spp was found in any of the 52 samples analyzed (Table 1).

According to information provided by the owners of the puppies, all specimens were collected within 2-5 days after the onset of the clinical signs. Among the 52 puppies, 7 had received parvovirus vaccine and 2 of them were considered CPV positive. Though the fecal samples of these 2 CPV-positive vaccinated puppies had not been collected during vaccine virus shedding (3-9 days post-vaccination), PCR was realized to confirm the infection with wild virus (CPV-

Table 1. Detection of CPV and Intestinal Parasitism in 52 Puppies (up to 6 months old) Showing Signs of Gastroenteritis.

Intestinal Parasites	CPV		
	Positive	Negative	Total
<i>Cystoisospora canis</i>	0	1	1
<i>Cystoisospora ohioensis</i>	0	1	1
<i>Giardia</i> spp	0	3	3
<i>Toxocara canis</i>	0	1	1
<i>Ancylostoma</i> spp	1	2	3
<i>C canis</i> + <i>C ohioensis</i>	1	0	1
<i>Ancylostoma</i> spp + <i>T canis</i>	0	1	1
<i>C ohioensis</i> + <i>Ancylostoma</i> spp + <i>T canis</i>	0	1	1
<i>Cryptosporidium</i> spp	0	0	0
Negative	16	24	40
Total	18	34	52

Table 2. Occurrence of Clinical Signs in Gastroenteritis Puppies (up to 6 months old) Tested for CPV and Parasitic Infection.

Laboratory Diagnosis	Clinical Signs														Total						
	V, A, L, HFD	V, A, L, HSD	V, A, L, SD	V, L, HFD	A, L, HSD	A, L, HFD	L, FD	L, SD	A, SD	A, FD	A, L, FD	V, SD	SD								
Only CPV																					
Positive	10	2	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	18	
Negative	15	0	0	0	0	2	0	0	3	1	1	1	1	1	1	1	0	0	7	34	
Only intestinal parasites																					
<i>Cystisporospora</i> spp	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
<i>Giardia</i> spp	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	3
<i>Toxocara canis</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
<i>Ancylostoma</i> spp	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
<i>Ancylostoma</i> spp + <i>T canis</i>	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>C ohioensis</i> + <i>Ancylostoma</i> spp + <i>T canis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Negative	22	2	1	3	0	0	2	1	1	0	0	0	0	1	0	0	1	0	0	7	40
CPV+ intestinal parasites																					
CPV + <i>Ancylostoma</i> spp	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
CPV + <i>C canis</i> + <i>C ohioensis</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1

V = vomiting; A = anorexia; L = lethargy; F = fluid; H = hemorrhagic; S = soft; D = diarrhea.

2b) as these 2 puppies were vaccinated with old type virus (CPV-2 [CPV-2b]).

The classical clinical signs of CPV enteritis (vomiting, anorexia, lethargy, and hemorrhagic fluid diarrhea) could be observed in 10 of the 18 CPV-positive puppies and 15 of the 34 CPV-negative puppies. For the remaining 27 puppies (8 CPV positive and 19 CPV negative), the association of clinical signs was variable (Table 2).

Only 3 puppies with parasitic infection presented vomiting, anorexia, lethargy, and hemorrhagic fluid diarrhea. One puppy with concurrent infection (CPV + *Ancylostoma* spp) presented more severe clinical signs while another (CPV + *C canis* + *C ohioensis*) showed only mild soft diarrhea (Table 2).

DISCUSSION

Although the presence of intestinal parasitism as predisposing or aggravating factor for CPV infection is reported in the literature,^{11,17,18} the concomitant CPV and parasitic infection could be observed in only 2 puppies (3.8%), and could not be related to the severity of the enteric illness.

The occurrence of intestinal parasites (23%) was lower than demonstrated by other authors in Brazil (from 37.2% to 45.3%) that also used only one fecal sample for parasitic diagnosis.⁶⁻⁹ An important aspect that could explain this finding is that all samples in this survey were obtained from diarrheic puppies, in contrast to other studies which used fecal specimens from non-diarrheic dogs.^{6,19} It has further been reported that the examination of 2 specimens can increase the sensitivity of parasitological methods from 76% to 92%, especially to detect *G duodenalis*.¹²

Moreover, all the specimens were from household dogs, reducing the exposure to intestinal parasites, despite the possibility of transmammary and transplacental transmission in young puppies.²⁰ It is important to remember that in spite of the aggression existing in the parasite-host interaction, the parasitic burden and the sensitivity of the host are determinant elements in the clinical evolution of the infection and that it varies individually.²¹

Parvovirus enteritis can be controlled by vaccination but the detection of CPV in 34.6% from the 52 samples in this study confirm that CPV is still circulating in Rio de Janeiro as we demonstrated before.^{2,12}

The enteric illness is a common finding in veterinary practice and these results show that in cases of hemorrhagic or nonhemorrhagic diarrhea, with or without vomiting, lethargy, or anorexia, the diagnosis of CPV and intestinal parasites infection should be included in the differential diagnosis and therefore confirmed by laboratory methods.

As some of the parasites found in this survey are considered zoonotic, veterinarians should play an important role increasing the control of gastrointestinal parasitic infections. For this, laboratory tests are essential for a correct diagnosis and the design of preventive strategies.^{14,18}

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