

## DETECTION OF VIRAL PARTICLES IN FECES OF YOUNG DOGS AND THEIR RELATIONSHIP WITH CLINICAL SIGNS

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Submitted: June 22, 1998; Returned to authors for corrections: September 29, 1998; Approved: August 09, 1999

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### ABSTRACT

Gross and light microscopic studies of 100 stool specimens of young dogs were carried out. Viral particles were detected in 31% of the analyzed samples using negative contrast electron microscopic diagnostic technique. *Parvo-like* virus, *corona-like* virus and other non-identified particles were observed in 17%, 7% and 2% of the samples, respectively. *Parvo-like* and *corona-like* viruses were found together in 5% of the samples. More than half (58.82%) of the positive *parvo-like* virus specimens were from dogs aged between 6 weeks and 6 months. 42.85% of the *corona-like* virus positive samples were detected in dogs between 6 weeks and 6 months and a similar percentage was found in dogs older than six months of age. Dual infections with *parvo-like* and *corona-like* viruses were observed in 5% of the samples. Unidentified *virus-like* particles were found in two specimens. 80.63% of the samples containing viral particles were obtained from dogs with diarrhea.

**Key words:** *Parvo-like* virus, *corona-like* virus, diarrhea, dogs, electron microscopy

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### INTRODUCTION

Parvovirus (CPV) and coronavirus (CCV) have clearly been established as causes of enteritis and diarrhea in dogs and cats (5, 8, 11, 13, 15). Other viruses, like rotavirus, astrovirus, adenovirus, paramixovirus, calicivirus and picornavirus have been also observed in canine feces (4, 6, 9, 13, 17). Some of these viruses were found with equal frequency in normal feces and feces from animals with diarrhea, and have not been established as enteropathogens yet (11). Rotavirus has been recognized as one of the most important cause of neonatal diarrhea in humans, cattle, swine, sheep and mice (2, 11). In dogs rotavirus has

been associated with a neonatal transitory diarrhea with lethal course in a few of them (9). An incidence of anti-rotavirus antibodies has been demonstrated in 79% to 86% of the animals (9, 11). These findings suggest that many animals present subclinical infections during their lives (9).

The incidence of these viruses in Argentina is not known. However, in a preliminary study carried out by the authors on 42 samples of canine feces, no rotavirus particle was detected (1).

The most frequent diagnostic methods for titration of viruses in feces are hemagglutination tests (HA) and enzyme-linked immunosorbent assays (ELISA). CPV, CCV and other enteric viruses can be diagnosed

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by isolation from feces, detection by electron microscopy and identification by immune electron microscopy (3, 11).

Serologic tests to detect anti-rotavirus antibodies have been used in research but are not currently available for clinical diagnosis (11).

The objective of the present paper was to relate the clinical features of diarrhea of young dogs with the recognition of viral particles in the feces, through electron transmission microscopy. In order to establish other causes of diarrhea examination of feces through optical microscopy were also carried out.

## MATERIALS AND METHODS

**Fecal samples.** The fecal samples (n: 100) were obtained from dogs submitted to the Small Animal Clinic of La Plata National University for clinical examination. Sixty two had diarrhea as the main problem.

The animals aged between 15 days and 12 months. Dogs were submitted to a complete clinical examination. These findings as well as vaccination status were registered in previously designed protocols.

**Gross and microscopic examination of fecal samples.** Gross examination of the feces was performed taking into account colour, consistency, presence of mucus and fresh blood. The light microscopy included the evaluation of fatty globules by Sudan III in 95% ethylic alcohol (16). A smear was stained with one drop of New Methylen Blue to count leukocytes, other cells and fibers. Direct coproparasitological examination was also included for identification of tapeworms, ascarids, hookworms, coccidia, oocysts and flagellated trophozoites of giardias.

**Negative contrast electron microscopy (1).** 1 ml of each fecal sample was homogenized with 10 ml of phosphate buffered saline (PBS) pH 7.2, and centrifuged at 8,000 g during 15 minutes at 4°C. The sediment was discarded and a second centrifugation with a Beckman L7-65 (rotor 70.1 Ti) ultracentrifuge at 45,000 r.p.m. during two hours at 4°C was performed. The sediment was resuspended 1:1 with PBS, and one drop was placed on a cooper grid (carbon substrate), and allowed to stand one minute. The excess was removed with a piece of filter paper. Two grids were negatively stained with 2% phosphotungstic acid, pH 7 during 1 minute, and

examined with a JEM 1200 EX II (Jeol) electron microscope. Each grid was scanned among 15 minutes before recorded as negative. The morphological identification was done according to the morphology and size of the viral particles (14).

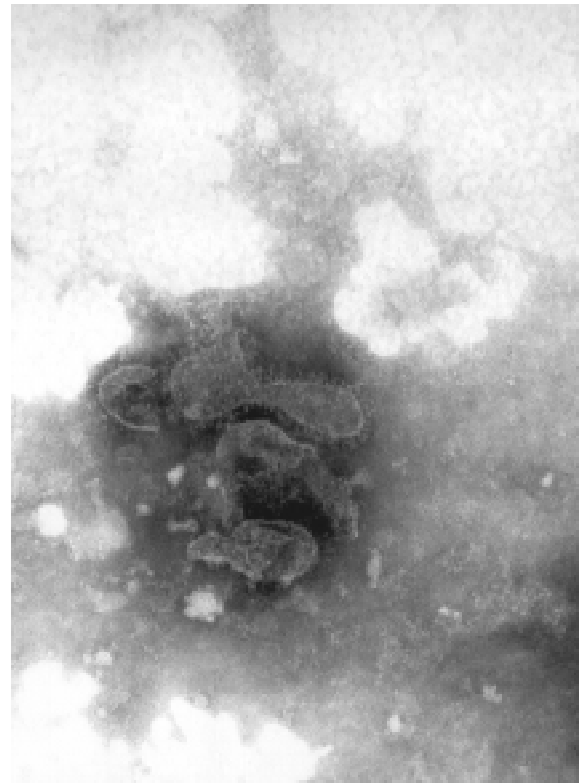
## RESULTS

The dogs were divided in three different groups according to their ages: Group 1: 1week to 6 weeks; Group 2: 6 weeks to 6 months and Group 3: 6 months to 12 months.

Viruses were detected in 31 fecal samples (31%). *CCV-like* viruses (Fig 1.) were found in 12 samples (12%), and *CPV-like* viruses (Fig. 2) were observed in 17 samples (17%).

The distribution of the viral findings according to the age of the animals is shown in Table 1.

The highest frequency of viruses was observed in animals of Group 2: 10 positive samples (10%) for *CPV-like* viruses and 3 (3%) for *CCV-like* viruses.

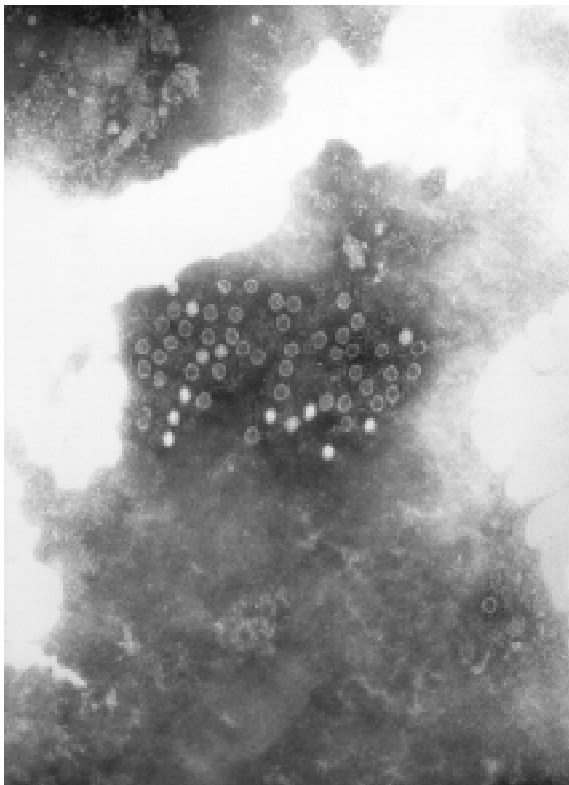


**Figure 1** – Electron microscopy showing a *corona-like* virus, x 222K (negative strain).

**Table 1** – *Parvo-virus* like (CPV) and *corona-virus* like (CCV) particles in fecal samples of dogs with and without diarrhea.

Virus detected	Ages of dogs												Total %
	1w-6w				6w-6m				6m-12m				
	Normal		Diarrhea		Normal		Diarrhea		Normal		Diarrhea		
n	%	n	%	n	%	n	%	n	%	n	%	n	%
CPV-L	1	1%	5	5%	1	1%	9	9%	–	–	1	1%	17%
CCV-L	1	1%	–	–	1	1%	2	2%	–	–	3	3%	7%
CPV/CCV-L	–	–	–	–	1	1%	4	4%	–	–	–	–	5%
Unidentified	–	–	–	–	1	1%	1	1%	–	–	–	–	2%
Total	2	2%	5	5%	4	4%	16	16%	–	–	4	4%	31%

W: week- m: month

**Figure 2** – Electron microscopy showing a group of *parvo-like* virus, x170 K (negative strain).

Five dual infections (5%) with *CPV-like* and *CCV-like* viruses were also observed. They were detected in animals aged between 6 weeks and 6 months. Unidentified virus-like particles were found in two specimens (2%).

**Table 2** – Fecal consistency and body temperature of 31 dogs with CPV-like and CCV-like viral particles in their feces.

Consistency	<i>Parvo-like</i>	<i>Corona-like</i>	<i>Parvo &amp; corona-like</i>	unidentified
Normal	2	2	1	1
Soft	4	3	–	1
Liquid	11	2	4	–
Body temperature				
Normal T°	15	5	2	1
Fever	2	2	3	1

The relationship between feces consistency, body temperature of the dogs and presence of viral particles is presented in Table 2.

The microscopic examination indicated that eleven positive fecal samples contained also a few nematod eggs (ascarids and hookworms). Only one sample had tapeworm eggs, and four presented giardias. Fatty globules and leucocytes counts were normal in all samples. Blood was found in 11 of the positive samples and 7 contained mucus.

None of the dogs had been immunized against canine parvovirus or corona-virus.

## DISCUSSION

The identification of the CPV in feces can be carried out only during the elimination period of the viruses, which occurs between the 3<sup>rd</sup> and 9<sup>th</sup> day of infection (7). As serological or molecular studies were

not done, the low frequency observed in our study may be artificial.

Eighty point sixty three percent of the samples that presented viral particles belonged to dogs with soft (25.80%) and liquid (54.83%) feces. As fatty globules and leukocytes counts were normal, it can be assumed that diarrhea was not a consequence of maldigestion syndromes or bacterial infection (17).

Among 17 fecal samples with *CPV-like* particles, 2 presented normal consistency and 15 were liquid, six of them were also positive for ascarids, and 1 for flagellated trophozoites of giardias because they were in a few number, it was assured that they didn't produce disease.

Two normal, three soft and two liquid feces were found in the *CCV-like* positive fecal samples. This is in agreement with the findings by Pollock and Carmichael (9) who concluded that probably there is not a narrow association between *CCV* and severe gastroenteritis in dogs.

The results of the present study, showing a low incidence of *CCV-like* infection, may be artificial. This may be due to some problems associated with identification of this virus. The most important characteristic of this virus is the fragile envelope, whose integrity can be affected by the length of storage and by the handling of the sample. Since there was a limited control over the time of storage of the samples, a consequent loss in integrity of *CCV* could have interfered with the unequivocal identification of the virus (14).

Two dogs with *CPV-like* particles in their feces were febrile and 9 of them were depressed and anorexic. These findings are coincident with the results reported by Swango (12), who found that depression and fever in *CPV* or *CCV* infected animals are common.

Dual presence of *CPV-like* and *CCV-like* viruses occurred in 5 dogs (16.12% of the positive samples). This result disagrees with those reported by Scherding (11), who said that the dual infection occurs in up to 25 per cent of cases of canine viral enteritis. The dogs with dual infection were more seriously ill than those infected with *CPV-like* or *CCV-like* viruses alone.

Fresh blood was found in 29.41% of the fecal samples containing *CPV-like* particles. This finding is not coincident with our preliminary report (18.60%) (1) and with Pollock and Carmichael (9), who found bloody diarrhea in 50% of the *CPV* infected dogs.

A high percentage of positive samples for *CPV-*

*like* particles was found in puppies under 5 months of age (52.94%), confirming the age susceptibility to *CPV-like* infection described by Hammond and Timoney (6). Dehydration and acidosis develop more quickly in puppies than in older animals. The puppies of this study experienced a severe illness, were anorectic and depressed, and their feces were liquid.

In the present study, *CPV-like* particles were found to be more closely associated with canine gastroenteritis than *CCV-like* particles. This is coincident with the results of Roseto *et al.* (10), who found a prevalence of this virus associated with gastroenteritis.

In this study, rotavirus-like particles were not identified in the samples. Further surveys are needed to investigate the prevalence of this virus.

## RESUMO

### Detecção de partículas virais em fezes de cães jovens e sua relação com sinais clínicos

Foram analisadas macroscópica e microscopicamente 100 amostras de fezes de cães jovens colhidas em ambulatório clínico. Através de microscopia eletrônica de contraste negativo, foram detectadas partículas virais em 31% das amostras de fezes examinadas. Do total de amostras onde foram detectadas partículas virais, 17% continham partículas *parvo-like*, 7% continham partículas *corona-like* vírus e 2% continham partículas com morfologia não característica. Em 5% das amostras foi detectada a presença simultânea de partículas *parvo-like* e *corona-like*. Do total de amostras de fezes contendo partículas *parvo-like*, 58,82% eram de cães com 6 semanas a 6 meses de idade, o que também foi observado em 42,85% das amostras de fezes contendo partículas *corona-like* foram cães entre 6 semanas e 6 meses e um número semelhante foi encontrado em cães de mais de 6 semanas de idade. Uma dupla infecção de *parvo-like* vírus e *corona-like* vírus, foi confirmada em 5% dos espécimens. Partículas “*virus-like*” não identificadas foram encontradas em 2 indivíduos Das amostras com identificação de partículas virais, 80,64% pertenceram a cães com diarreia.

**Palavras-chave:** parvo-like vírus, corona-like vírus, diarreia, cães, microscopia eletrônica

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