

## Canine parvovirus infection in puppies with gastroenteritis in Niterói, Rio de Janeiro, Brazil from 1995 to 1997 \*

### Infecção pelo parvovírus canino em filhotes com gastroenterite em Niterói, Rio de Janeiro, Brasil de 1995 a 1997

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#### SUMMARY

Fecal samples from puppies with gastroenteritis less than 7 months old were examined for canine parvovirus infection (CPV-2) by hemagglutination (HA) and subsequent hemagglutination-inhibition (HI) tests. Forty of the 79 samples collected from April 1995 to June 1997 were found to be positive. About 70% of these samples were from 2 to 4 months old puppies, age in which they are at increased risk of developing CPV-2 infection, despite of vaccination. No seasonal distribution of canine parvovirus cases was found and it was supported by the results of a retrospective study performed at PolVet-UFF, which showed that gastroenteritis cases occurred throughout the year, for a six-year period (1991-97) in Niterói, Rio de Janeiro.

**UNITERMS:** Canine parvovirus; Gastroenteritis; Haemagglutination tests.

#### INTRODUCTION

The first cases of enteritis and myocarditis caused by canine parvovirus (CPV-2) were recognized in the United States in 1978. Since then, CPV-2 infection had spread rapidly throughout the world<sup>2,3</sup>.

Initial outbreaks of parvovirus in dogs were characterized by high morbidity and mortality in all age groups. In Brazil, the first cases of hemorrhagic enteritis were first observed in Campinas and lately detected in São Paulo during 1979-1980<sup>1,9</sup>. After that, CPV-2 infections have also been diagnosed in Rio Grande do Sul<sup>14</sup>.

Parvovirus infection in dogs causes an acute disease characterized by severe vomiting and diarrhea. Disease is most commonly seen in puppies from 6 weeks to 6 months of age. Most adult dogs have become immune through vaccination or natural infection<sup>3</sup>.

Infected dogs can shed more than 10<sup>9</sup> tissue culture

infectious dose (TCID<sub>50</sub>) of CPV-2 per gram of feces. Consequently feces constitute the major source of infection for susceptible animals and they also represent the most useful material for diagnosis<sup>3,4</sup>.

Several methods have been used to detect CPV-2 in fecal samples. Electron microscopy by negative staining, virus isolation in cell cultures and enzyme immunoassay<sup>7,12,13,18</sup>. But, for rapid diagnosis, the most frequently used is the hemagglutination (HA) assay followed by hemagglutination-inhibition (HI) test with a CPV-2-specific antiserum<sup>4,7,12,17</sup>.

A preliminary study conducted in São Paulo by Mehnert *et al.*<sup>11</sup>, in order to identify the viral agents that cause hemorrhagic gastroenteritis in dogs, showed that canine parvovirus was responsible for the most of the cases.

The purpose of the present study is to determine the occurrence of parvovirus infection in puppies suffering from gastroenteritis in Niterói, Rio de Janeiro, during a two-year period.

\* Supported by CNPq, Pfizer Saúde Animal.

## MATERIAL AND METHOD

The seventy-nine fecal samples in this study collected from April 1995 to June 1997 from puppies less than 7 months of age with gastroenteritis were obtained from different sources. Fifty-one were from Policlínica Veterinária, Universidade Federal Fluminense (PolVet), 20 samples were from a private owned Animal Hospital, Veterinária das Meninas (Vet Men), both located in Niterói city. Another 8 samples submitted for diagnosis were from dogs seen by practitioners at Rio de Janeiro city.

Approximately 10% of fecal material suspensions were prepared in Hank's balanced salt solution. After vortex and centrifugation the supernatants were treated with 1/10 volume of chloroform as described previously<sup>4</sup>.

For the HA test, serial 2-fold dilutions of the 10% fecal suspensions were made in BABS buffer pH 9.0 (1.5M NaCl, 0.5M H<sub>3</sub>BO<sub>3</sub>, 1.0M NaOH, 0.2% BSA). Then, an equal volume (50 µl) of 0.5% porcine or rhesus monkey erythrocytes diluted in VAD buffer pH 6.0 (0.15M NaCl, 0.3M Na<sub>2</sub>HPO<sub>4</sub>, 0.15M NaH<sub>2</sub>PO<sub>4</sub>) were added and incubated at 4°C. Tests were read after the erythrocyte controls had settled completely. Samples with HA titers ≤ 8 were considered negative. Samples with HA titers ≥ 16 were confirmed as positive in the HI test<sup>16,17</sup>.

The specific anti-CPV-2 serum used in HI test was obtained after immunizing adult guinea pigs with Parvoguard<sup>®</sup> vaccine (Solvay Saúde Animal Ltda.)<sup>6</sup>. Initially, serum sample was heated at 56°C for 30 minutes, treated with 25% Kaolin and then adsorbed with 50% erythrocyte suspension. Serial 2-fold dilutions of serum in BABS were made in microplates, and an equal volume (25 µl) of 10% fecal suspensions (diluted to contain 8 UHA/50 µl) was added to each well. After incubation, 50 µl of 0.5% erythrocyte suspension diluted in VAD buffer pH 6.0 was added. HI endpoints were determined after an overnight incubation period at 4°C. Samples were considered positive when the HA reaction was inhibited by the antiserum. Each test series included appropriate erythrocyte, antigen (vaccine) and serum controls<sup>16</sup>.

From a total of 79 samples submitted for diagnosis, 39 were from mongrel puppies, 7 from Doberman pinschers, and 4 from Rottweilers. Another 20 samples were distributed among the following breeds: German shepherd (7), Fila (4), Boxer (3), Husky (3), Cocker spaniel (2), Dachshund (2), Poodle (2), Yorkshire (2), Basenji (1), Beagle (1), Setter (1) and Terrier (1).

## RESULTS

Fecal samples from 42 male and 37 female puppies suffering from diarrhea and vomiting were tested for the

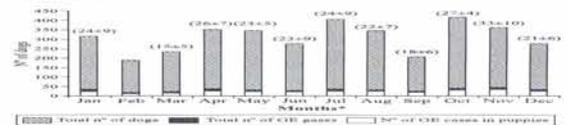
presence of CPV-2 by HA/HI tests, and 40 (23 males and 17 females) of these 79 samples were positive. As shown in Tab. 1, the majority of the 79 samples were collected from puppies within two, three or four days of the onset of the symptoms. At least 50% of the samples tested within this period were positive. Fifty-seven (72%) of the 79 samples were from animals ranging from 2 to 4 months of age, and 33 samples (58%) were positive (Tab. 2).

The annual distribution of canine parvovirus cases from April 1995 to June 1997 was examined. From 20 samples received in 1995, 18 (90%) were positive. Among 39 samples tested in 1996, CPV-2 was confirmed for 17 (44%) while in 1997 five (25%) of the 20 samples were positive.

The distribution of the number of gastroenteritis cases in relation to the total number of dogs attending at PolVet from January 1991 to December 1997 is shown in Fig. 1. Gastroenteritis could be diagnosed in about 10% of the dogs

**Table 1**

Distribution of confirmed CPV-2 cases in relation to the day of disease in Niterói from 1995 to 1997.



\* Not available for three animals.

**Table 2**

Distribution of confirmed CPV-2 cases in relation to the age of the puppies in Niterói from 1995 to 1997.

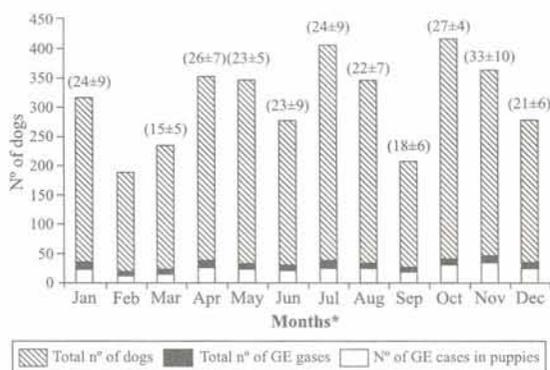
Age (months)	1	2	3	4	5	6	Total
N° of positive samples / N° of samples tested	3/6	10/18	12/20	11/19	2/8	2/8	40/79

**Table 3**

CPV-2 positive cases detected in vaccinated puppies in Niterói from 1995 to 1997.

Case	Breed	Age (months)	N° of days between vaccination and onset of the disease	
			1 <sup>st</sup> dose	2 <sup>nd</sup> dose
1	Mongrel	2	4	—
2	Siberian husky	3	8	—
3	Mongrel	2	24	—
4	Rottweiler	3	37	—
5	Boxer	3	49	—
6	Doberman pinscher	4	61	—
7	Yorkshire	5	83	—
8	Doberman pinscher	5	91	—
9	Cocker spaniel	4	NK*	1
10	Rottweiler	4	47	7
11	Fila	3	48	7
12	Fila	3	50	22

\* Not known.



**Figure 1**

Distribution of the total number of gastroenteritis (GE) cases and the total number of dogs attending at PolVet-UFF in the period of 1991 to 1997. \* Average monthly values of GE cases from 1991-1997. Number in parenthesis means  $X \pm \sigma$  of the GE cases in puppies.

throughout the year and at least 70% of these cases occurred in puppies less than 7 months of age.

From the 40 CPV-2 positive puppies, 27 had not been vaccinated and for another one this data was not known. Among 12 positive puppies that had been vaccinated, 8 had received only the first dose and in two of them (cases 1 and 2) enteritis symptoms began 4 and 8 days after vaccination (Tab. 3). A further 4 puppies were given two doses of vaccine, and three of them received the second dose within 7 days before the onset of the disease (cases 9, 10, 11). One of these was a Rottweiler that died.

## DISCUSSION

Canine parvovirus emerged as a new pathogen of dogs in the late 1970s. Initially seen as epidemic disease in all dogs, parvoviral enteritis now may be controlled by vaccination. Disease is commonly seen in 1 to 6 months old puppies due to vaccination failures because the interference of maternal antibodies<sup>3,15</sup>.

In the present study we were able to demonstrate, by HA/HI tests, canine parvovirus infection in 44% of puppies with gastroenteritis during a two-year period. This high

proportion was probably due to the age range of the puppies studied (2 – 4 months). At this age vaccination may not be completed<sup>15</sup>, and the majority of these puppies had not been vaccinated.

As shown by our results, 12 of the positive puppies had received one or two doses of vaccine. Using the HA assay it is not possible to discriminate between field and vaccine virus, and it is known that both vaccine and wild CPV-2 can be detected in fecal samples by this test from 3 to 9 days after oral infection<sup>4,5</sup>. In 7 of these vaccinated puppies, the finding of CPV-2 does not really mean vaccine virus shedding, because the interval between the date of vaccination and days of disease was longer than 15 days. In 5 positive puppies the HA result could be due to the detection of vaccine virus because the fecal samples were collected from 1 to 8 days after vaccination.

Certain breeds like Rottweiler and Doberman pinscher are reported to be more susceptible to the development of parvovirus disease<sup>8</sup>. This could not be confirmed in our study because of the low number of samples received from Rottweilers and Doberman pinschers.

In a study performed in Canada, the incidence of CPV-2 infection peaked in the months of July, August and September, during the warm season<sup>10</sup>. No seasonal variation of CPV-2 infection in Niterói was apparent but the low number of samples received during a two-year period means this is not definitive. The data from PolVet-UFF during 1991 to 1997 show that gastroenteritis cases occur throughout the year without a clearly defined seasonality and we detected parvovirus infection throughout the study period.

Our results confirm that CPV-2 infection in puppies is a significant cause of morbidity in the city of Niterói, Rio de Janeiro.

## ACKNOWLEDGEMENTS

The authors wish to thank Drs. Cristiane Oliveira Milward and Mariluci Gomes Espariz (Veterinária das Meninas) who generously provided fecal samples for testing. The authors also wish to thank Dr. David Brown (Virus Reference Division, CPHL, London) for critical reading of the manuscript.

## RESUMO

Amostras fecais de cães com gastroenterite, até 6 meses de idade, foram testadas para a presença do parvovírus canino (CPV-2) pela reação de hemaglutinação (HA) e confirmadas pela reação de inibição da hemaglutinação (HI). Quarenta das 79 amostras, recebidas no período de abril de 1995 a junho de 1997, foram consideradas positivas. Aproximadamente 70% destas amostras foram obtidas de animais entre 2 e 4 meses de idade, época em que o risco de contraírem a infecção pelo CPV-2 é alto apesar da vacinação. Nenhuma variação sazonal da infecção pelo parvovírus canino pôde ser observada, e um estudo retrospectivo realizado na PolVet – UFF mostrou que em um período de 6 anos (1991-97), casos de gastroenterite ocorreram durante todos os anos em Niterói, sem uma sazonalidade definida.

**UNITERMOS:** Parvovírus canino; Gastroenterite; Reações de hemaglutinação.

CUBEL-GARCIA, R.C.N.; VIANA-PINTO, A.M.; COSTA, A.P.; MACIEL, B.M.; OLIVEIRA, L.H.S.; NASCIMENTO, J.P.; SANTOS, A.O.; NOBRE E CASTRO, M.C.; WILLI, L.M.V.; LABARTHE, N.V. Canine parvovirus infection in puppies with gastroenteritis in Niterói, Rio de Janeiro, Brazil from 1995 to 1997. **Braz. J. vet. Res. anim. Sci.**, São Paulo, v. 37, n. 2, p. 132-135, 2000.

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Received: 15/12/1998

Accepted: 03/09/1999