

## DETECTION OF ROTAVIRUS IN DOGS WITH DIARRHEA IN BRAZIL

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### ABSTRACT

Rotavirus was detected by the enzyme-linked immunosorbent assay (ELISA) in the faeces of a diarrheic dog. Virus particles with morphology typical of rotavirus were visualized by direct electron microscopy. This sample was subsequently tested for the four main human serotypes (G1-G4), by ELISA with monoclonal antibodies. G genotyping was attempted by RT-PCR using G1-G6 and G8-G11 primers but no positive results could be yielded. Also using RT-PCR it was possible to characterize this canine strain as belonging to P[3] genotype. This is the first canine rotavirus detected in Brazil.

**Key words:** rotavirus, diarrheic dog, genotype G, genotype P, Belém- Brazil

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### INTRODUCTION

Rotaviruses are the major cause of acute viral gastroenteritis in infants and young children, as well as in young animals of several species, including strains of bovine, equine, porcine and canine origin (16).

Rotavirus is classified in the family *Reoviridae*, genus *Rotavirus*, comprising five species (*Rotavirus A* to *E*) and two tentative species (*Rotavirus F* and *G*). *Rotavirus A* (RV-A) is further classified into G and P serotypes/genotypes according to the two external capsid proteins, VP7 and VP4, respectively (34).

Rotavirus infections in dogs have already been reported elsewhere (4,6,9). Viral particles with morphology typical of rotavirus were detected by electron microscopy (EM) in the faeces from a 12-week-old pup with nonfatal diarrhea (6). Other authors (4) have associated rotavirus infection with a fatal neonatal diarrhea in a 3-day-old pup, where the virus was detected by negative contrast EM examination of intestinal contents and, thereafter, the agent was successfully propagated in MDCK (Madin-Darby canine kidney) cells. Similar results were obtained

by Fulton *et al.* (9), who observed diarrhea in several litters of pups (< 1 week old) from the same kennel. In a 2-day-old pup that died from diarrhea, rotavirus particles were detected by EM in specimen prepared from the intestinal homogenate. Roseto *et al.* (26), examining stool samples from apparently normal dogs randomly caught on the sidewalks of Paris, France, detected 2 (3.6%) rotavirus-positive faecal specimens by EM. In addition, Hoshino *et al.* (15), reported the detection of rotavirus, by EM, in fecal samples from an apparently normal, one-year old beagle. This agent was subsequently propagated in cell cultures and characterized through comparison with rotaviruses from other species. By plaque reduction neutralization assay, a two-way antigenic relationship was found between this virus and simian (rhesus MMU 18006 and SA-11) rotaviruses and a one-way antigenic relationship with feline (Taka), bovine (NCDV), and porcine (OSU) rotaviruses. Dogs experimentally infected with a human rotavirus (32, 33) did not present diarrhoea, although rotavirus antibodies have been detected and viral particles were visualized by TEM in their faeces. These studies indicate that dogs may be infected with rotaviruses from different species and that transmission may occur through direct contact. These

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findings also indicate that dogs may have a role in the transmission and dissemination of rotavirus between humans and other species. The environmental contamination by dogs' stools represents a serious sanitary problem in many urban and suburban areas, and, since the detection of rotavirus in stools from these animals has been previously reported (26), this may be of epidemiological importance.

The present report deals with the detection of rotavirus in dogs from Belém, PA and Botucatu, SP, Brazil.

## MATERIALS AND METHODS

### Fecal specimens

A total of 70 faecal specimens (one per dog) were collected from diarrheic and asymptomatic dogs from April to July 1996. Twenty-nine samples were obtained in the Veterinary Hospital of the "Faculdade de Ciências Agrárias do Pará (FCAP)", Belém, Pará, and forty-one samples from the Veterinary Hospital of the "Faculdade de Medicina Veterinária e Zootecnia (FMVZ)", Botucatu, São Paulo, Brazil. Clinical data were obtained from forms filled out by the attending veterinarians. Dogs were from different races. In relation to the age, most of them had one to five months, and male dogs (60.6 %) predominated over female dogs (39.4%).

### Electron microscopy (EM)

The examination of fecal samples was performed essentially as previously described (1). Briefly, crude faecal specimens were mixed with distilled water and applied onto a carbon-collodium coated 400-mesh copper grid. The grid was subsequently dried and the preparation stained with one drop of 2% phosphotungstic acid (PTA) pH 7.2. The grid was then examined by transmission electron microscopy (TEM), in a Zeiss EM900 electron microscopy model, at 80 KV. Virus particles were sought in at least five grid squares and identification was made following the criteria proposed by Flewett and Woode (8).

### Enzyme-linked immunosorbent assay

All specimens were tested by the EIARA ELISA, developed at Oswaldo Cruz Foundation, Rio de Janeiro, Brazil (25). This test allows the detection of both group A rotaviruses and adenoviruses. The stools were also tested by a commercial DAKOPATTS ELISA kit (Copenhagen, Denmark), as previously described (7). G serotyping was performed using monoclonal antibodies against each of four epidemiologically important human G serotypes (G1, G2, G3, and G4), essentially as reported by Taniguchi *et al.* (28).

### Polyacrylamide gel electrophoresis (PAGE)

Electrophoresis of deproteinized rotavirus dsRNA was carried out through a 5% polyacrylamide gel, using discontinuous buffer system, as recommended by Laemmli (17).

### Reverse transcription-polymerase chain reaction (RT-PCR)

G and P genotyping was performed in two steps, RT-PCR and semi-nested PCR as described previously (10,11). The second amplification was carried out for G typing using two sets of primers specific for human genotypes G1, G2, G3, G4 and G9 (3,11); and one set specific for porcine genotypes G5 and G11 and bovine genotypes G6, G8 and G10 (12). For P typing, bovine, porcine and human rotaviruses primers, specific for VP4 genotypes P[1], P[3], P[4], P[5], P[6], P[7], P[8], P[9], P[10] and P[11] were used (10,13,18).

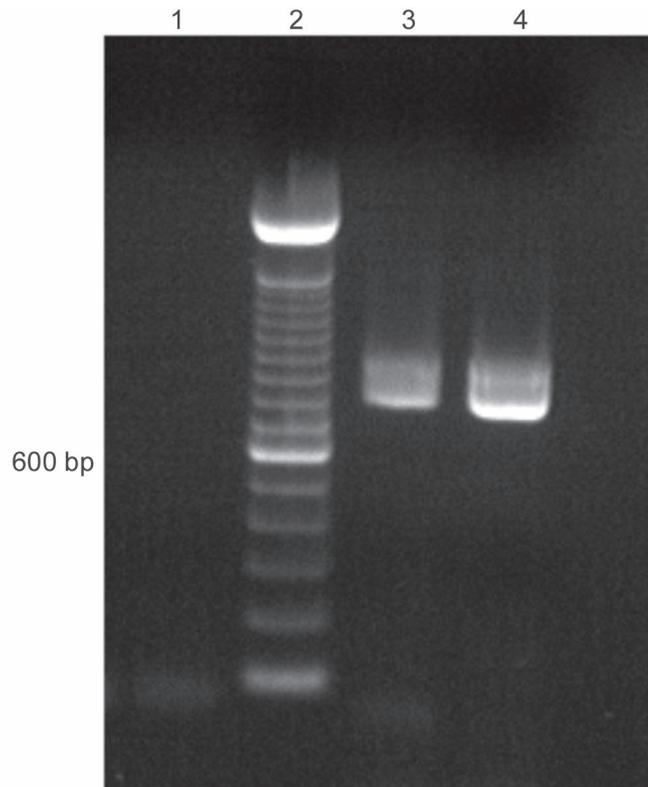
## RESULTS

Rotavirus particles measuring approximately 70nm in diameter were observed in faeces from one (3.0%) of the 33 specimens examined by TEM. Parvovirus, paramyxoviruses and coronavirus-like particles were also detected in 30.3%, 3.0% and 6.0%, respectively (14). Of 70 stools tested by ELISA, only one (1.4%) sample from a diarrheic case was positive for group A rotavirus by both ELISA methods, corresponding to the EM-positive sample. Sample was from a 8 month old S.R.D. dog, with the following symptoms: diarrhea, vomiting, dehydration and apathy. RNA electrophoresis of this sample showed a long electrophoretic profile. This pattern was classified as I2C IIW+ III2 IVL+ according to Moosai *et al.* (21), and as Ib IIa IIIc IVa electropherotype, as proposed by Lourenço *et al.* (19). This sample did not react with any of the monoclonal antibodies to serotypes G1, G2, G3 and G4. No amplification product was obtained using primers G1-G6, and G8-G11 in PCR. P typing however characterized this strain as genotype P[3] (Fig. 1).

## DISCUSSION

Rotaviruses have already been detected in faeces from dogs with diarrhoea (4,9). Using electron microscopy, Roseto *et al.* (26) detected rotavirus at rates (3.5%) lower than those observed for other pathogens such as parvoviruses (23.2%) and coronaviruses (12.5%). In our study, the results obtained by this technique were similar, since for rotavirus the positivity was 3.0%, for parvovirus and coronavirus-like 30.3% and 6.0% respectively (14). Serological studies (20), however, have shown that approximately 80% of the dogs have antibodies to rotavirus, suggesting that the majority of infections may be asymptomatic. One issue to be considered is that rotavirus antibodies detected in these dogs by immunofluorescence technique are group-specific, suggesting that interspecies infections may occur (2,30,32,35).

Most of the canine rotavirus samples are characterized as serotype G3 (24). Our sample did not react with any of the G MAbs, including G3 MAbs and was not amplified by RT-PCR for all the genotypes tested (G1-G6 and G8-G11), including two different G3 specific primers. This results indicates that



**Figure 1.** P genotyping of dog rotavirus sample. Lane 1: negative control (water); lane 2: 100 bp DNA ladder (Gibco); lane 3: HCR3 rotavirus strain; lane 4: dog sample. The sample produced amplified segment of 748bp, corresponding to genotype P[3].

our canine rotavirus strain may belong to a serotype different from G3.

P[3] genotype has already been identified in simian (strains Si/RRV), canine (Ca/K9, CaCu-1) and feline rotavirus specimens (strains Fe/FRV64, Fe/ Cat97) as well as in human samples (strains RO1845, HCR3) (5), including Brazilian human strains (31). Analysis conducted by Nakagomi and Nakagomi (23) showed, by RNA-RNA hybridization, that human strains HCR3 and Ro1845, are very closely related to each other and virtually indistinguishable from feline (strain Fe/FRV64) and canine (strains Ca/K9, CaCu-1) rotaviruses. Others authors also demonstrated this homology using different techniques (22,27,29). This suggests an interspecies transmission of rotavirus from cats and dogs to humans. However the impact of these samples on infant morbidity for rotavirus gastroenteritis has not yet been established.

To our knowledge this is the first report on the detection of a rotavirus of canine origin in Brazil. It would be rather important to further characterize the G type of this sample in order to compare it with other canine rotavirus strains worldwide.

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## RESUMO

### Deteção de rotavírus em cachorros com diarreia

Rotavírus foi detectado pela técnica imunoenzimática (ELISA) nas fezes de um cachorro que apresentava diarreia. Partículas virais com morfologia típica de rotavírus foram visualizadas por microscopia eletrônica direta. Essa amostra foi posteriormente testada para os quatro principais sorotipos humanos de rotavírus (G1–G4) por ELISA utilizando anticorpos monoclonais. Genotipagem para G foi realizada por RT-PCR usando "primers" específicos para G1-G6 e G8-G11. Nenhum resultado positivo foi obtido. Também utilizando RT-PCR, foi possível caracterizar essa amostra canina como genótipo P [3]. Este é o primeiro rotavírus canino descrito no Brasil.

**Palavras-chaves:** rotavirus, diarreia canina, genótipo G, genótipo P, Belém-Brasil.

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