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# Identification of co-infection by rotavirus and parvovirus in dogs with gastroenteritis in Mexico

A. Flores[a], J.S. Martínez-Castañeda[a,*], L. Bautista-Gómez[b], R. Fajardo[a], I. Quijano[c]

[a] Centro de Investigación y Estudios Avanzados en Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Carretera Toluca-Atlacomulco Km. 15.5, Toluca, Estado de México C.P. 50200, Mexico
[b] Centro Universitario Amecameca, Universidad Autónoma del Estado de México, Carretera Amecameca Ayapango Km. 2.5, Amecameca de Juárez C.P. 56900, Mexico
[c] Hospital Veterinario de Pequeñas Especies, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Jesús Carranza #203, Col. Universidad, Toluca, México, Mexico

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ABSTRACT

This is the first report on circulating canine rotavirus in Mexico. Fifty samples from dogs with gastroenteritis were analyzed used PCR and RT-PCR in order to identify parvovirus and rotavirus, respectively; 7% of dogs were infected with rotavirus exclusively, while 14% were co-infected with both rotavirus and parvovirus; clinical signs in co-infected dogs were more severe.



Infectious gastroenteritis are one of the main causes of dog hospitalization, etiological agents identification is a challenge for veterinarians, given that gastroenteritis etiology is caused by diverse pathogenic agents, mainly co-infections among virus or bacteria.[1–3]

Canine parvovirus (CPV-2) is a member of the *Parvoviridae* family, belonging to the *Protoparvovirus* genus and *Carnivore Protoparvovirus type 1* species. Diverse reports indicate that is the most diagnosed viral agent in gastroenteritis.[4,5] Over the past years, CPV-2 has developed new antigenic variants. In 1980 CPV-2 original strain was replaced by the variant designated type 2a (CPV-2a), in 1984 was identified CPV-2b and in 2001, CPV-2c was detected and reported in Italy. It is possible that CPV-2c is the predominant variant in numerous countries.[6]

Currently, there is some controversy over clinical characteristics of disease associated with these three variants; however, some authors suggest there are none clinical differences.[7]

Clinical signs of canine parvovirosis include fever, anorexia, lethargy, depression, vomiting, mucoid to

* *Corresponding author at*: Centro de Investigación y Estudios Avanzados en Salud Animal, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Carretera de Cuota Toluca-Atlacomulco, Kilómetro 15.5, Código Postal 50200, Toluca, Estado de México, Mexico.
E-mail: jsmartinezc@uaemex.mx (J. Martínez-Castañeda).
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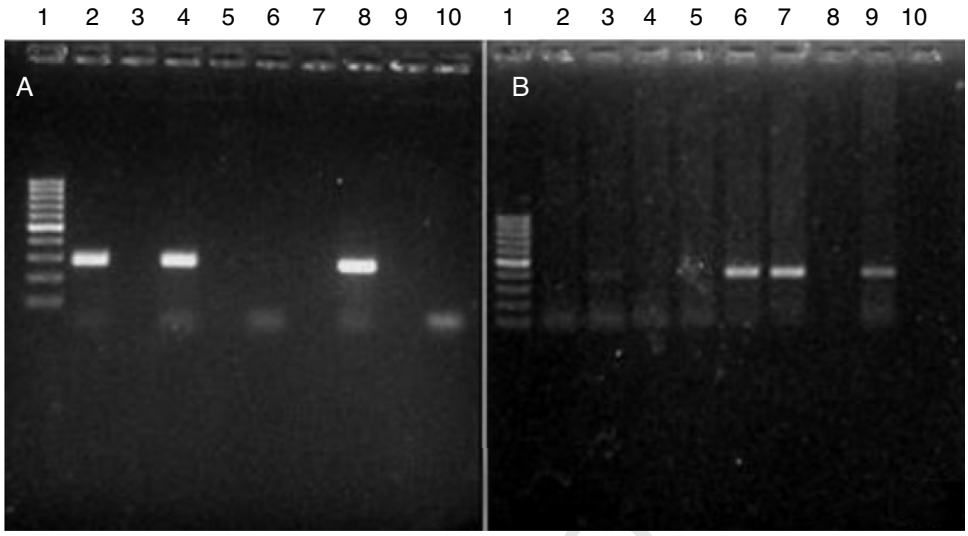


**Fig. 1 – (A) Parvovirus amplicons. The picture shows a 2.5× agarose gel containing PCR products (275 bp) from different samples. Lane 1: DNA length marker (gene ruler 100–1000 bp DNA Ladder plus, Fermentas); lanes 2, 4 and 8: CPV-positive samples. Lanes 6 and 10: CPV-negative samples. (B) Rotavirus amplicons (379 bp). Lane 1: DNA length marker (gene ruler 100–1000 bp DNA Ladder plus, Fermentas); lanes 3, 6, 7 and 9: CRV-positive samples; lanes 2, 4, 5, 8 and 10: CPV-negative samples.**

hemorrhagic diarrhea and sometimes leukopenia; however, some reports show parvovirus in dogs with atypical clinical signs, and the authors suggest that this is likely due to CPV-2 evolution.[8] On the other hand, the disease can vary depending on the patient; actually, CPV-2c can infect both pups and adults.[9]

A further factor implicated in variability of clinical profiles in parvovirus is association with other gastroenteric viruses such as canine distemper, canine coronavirus and canine rotavirus.

Actually, *rotaviruses* are classified as distinct members of the family reoviridae, genus *rotavirus*, comprising five species (A to E) and two tentative species (F and G). *Canine rotavirus* is a double-stranded RNA, non-enveloped virus that possesses a segmented genome and that is approximately 60–75 nm in diameter; few isolates of rotavirus have been reported in dogs, these have been classified as serotypes G3 and P5A, grouped into group A, rotavirus of this group cause neonatal diarrhea in human and many animal species; it has been demonstrated that direct interspecies transmission between heterologous strains are key mechanisms in generating rotavirus strain diversity in new hosts. Human infection for rotavirus of canine origin has been reported.[10,11]

Clinical signs of the disease include moderate enteritis, mainly in pups younger than two weeks old,[12,13] and it also causes lethargy, anorexia, fever, diarrhea and vomiting. Generally, patients recover within two weeks; however, there are reports of fatal severe enteritis in dogs under two weeks old.[14]

The presence of antibodies against rotavirus has been demonstrated in a high percentage of adult dogs (80%).[15] Rotavirus infection does not have pathognomonic clinical signs and most dogs can be asymptomatic to infection and occasionally signs can be confused with parvovirus, therefore, laboratory test for differential diagnoses are necessary.[3,13] In Mexico, it remains unknown whether rotavirus is circulating amongst canine populations and if it plays a primary role as etiologic agent in gastroenteritis.

From March through June 2015, we conducted non-probability sampling in dogs attending the small animal veterinary hospital of the Autonomous University of the State of Mexico (UAEM, by its acronym in Spanish). Fifty dogs of different ages, breeds, non-vaccinated dogs with gastroenteritis signs were selected. One rectal swab was taken from each dog to be used in PCR testing (parvovirus identification), while a second swab was taken for RT-PCR testing (rotavirus identification). Swabs were stored at −80 °C until processing.

The Rotateq vaccine (Merck, USA), which contains rotavirus serotypes G1, G2, G3 and P1A, was used as positive control, and in the case of parvovirus the Edo. Mex 1 isolate was used (previously identified in our laboratory and which corresponds to CPV2-c genotype).

In order to extract RNA from rotavirus, each swab was suspended in 200 μL of Diethylpyrocarbonate (DEPC 1%)-treated water and centrifuged 7 min at 5000 × *g*. Supernatant was processed using the GeneJET Viral DNA and RNA Purification kit (Thermoscientific, USA), following manufacturer's instructions.

Rectal swabs used for parvovirus detection were suspended in 500 μL of sterile water, centrifuged 1 min at 5000 × *g*. 200 μL of supernatant was processed for DNA extraction using the QIAamp DNA Mini Kit (Qiagen, USA) following the manufacturer's instructions.

In the case of rotavirus, two-step RT-PCR was performed for each of the samples, using the ImProm-II™ Reverse Transcription System kit (Promega, USA). In the first step, cDNA synthesis was accomplished. 3 μL of RNA of each purification plus 2 μL (0.5 μg/reaction) of primer dT was used in each reaction. All reactions were incubated at 70 °C for 15 min and



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