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Identification of Canine Parvovirus Antigenic Types Circulating in the Mexican Cat Population

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Abstract

Objectives. This study was aimed to investigate if canine parvovirus is infecting domestic cat populations in Mexico and determine the presence of different antigenic types of this virus affecting both healthy cats and cats with gastroenteritis.

Methods. Seventy-five cats were studied, 25 with gastroenteritis and 50 healthy, viral DNA was extracted, a stool sample from each cat was obtained using rectal swabs, 100 ng of DNA from each sample were used for the PCR reaction, cats positive for CPV-2 were identified utilizing this method, only samples of positive animals were amplified to obtain a complete sequence of VP2 gen and identified an antigenic type of CPV-2 infecting the cats.

Results. 60% of the cats with gastroenteritis and 22% of healthy cats were positive for Canine parvovirus; the variant 2c only was identified in healthy cats, while ten cats with gastroenteritis were infected by canine parvovirus 2a and type 2c.

Conclusions and relevance. This is the first report about variants of CPV-2 circulating in Mexican cats healthy and with gastroenteritis. The identification of canine parvovirus infection in cats is important because infected cats may contribute to the transmission of the disease to cats and dogs populations.

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Introduction

Diverse studies have demonstrated that *carnivore protoparvovirus 1* (canine parvovirus-2 CPV-2 common names) emerged as a variant of the feline panleukopenia virus (FPV). CPV-2 was isolated in 1978 in dogs with severe hemorrhagic gastroenteritis.[1] In 1979, a new variant of canine parvovirus emerged as CPV-2a, produced by four amino acid changes in CPV-2 (L87M, I101T, A300G, and D305Y). Shortly after that, in 1984, only one mutation (N426D) gave rise to another antigenic type, CPV-2b, which quickly spread worldwide.[2] In 2000, CPV-2c was identified, originating from a change in amino acid residue D426E.[3] It has been demonstrated that these last three antigenic variants of CPV-2 (a, b, and c) regained the ability to infect feline hosts, a capacity that had been lost in CPV-2. [4] The first report of natural infection by canine parvovirus in cats was made in 1993.[5] Cats infected with CPV-2 may or may not present clinical signs of disease. The virus can affect the thymus, mesenteric lymph nodes, duodenum, jejunum, ileum, and spleen; causing a relative lymphopenia, in contrast to the leukopenia caused by FPV in cats.[6] In an experimental study conducted with CPV-2c, the authors observed leukopenia in all the infected cats, and more severe clinical signs than those caused by other CPV variants. However, those signs were relatively less severe than those caused by FPV infection.[7] additionally, co-infections of different strains of CPV-2 with FPV have been reported.[8][9][10] There are currently no reports on whether or not canine parvovirus is in circulation amongst Mexican domestic cats. Therefore, the objective of this work was to identify the antigenic variants of CPV-2 circulating in Mexican cat populations.

Materials and methods

Sample collection and DNA extraction

Twenty-five cats with gastroenteritis clinical signs and 50 clinically healthy cats were sampled. The cats were randomly selected from veterinary hospitals. A stool sample from each cat was obtained using rectal swabs, which were suspended in nuclease-free water, and 200 µl of these homogenates were used for the extraction of DNA.
The procedure was performed using the QIAamp® DNA Stool DNA extraction kit (QIAGEN), following the manufacturer's instructions; 100 ng of DNA from each sample were used for the PCR reactions.

PCR Reactions

To identify cats positive for CPV-2, a pair of primers were designed, producing a 275 bp fragment of the VP2 gene in all samples. The characteristics of primers are ParvoInt2FB (5'-TCAAGCAGATGGTGATCCAAG-3') and ParvoInt2CR (5'-GGTACATTATTTAATGCAGTTA-3'), located at nucleotides 1107-1130 and 1360-1382, the sequence utilized was (GenBank accession number FJ0051962c).

PCR reactions were performed at a final volume of 50 µl. Under the following conditions: 2 µl of each primer (200 nM); 12.5 µl of GoTaq® Green Master Mix (Promega, USA), which contains DNA polymerase, reaction Buffer (pH 8.5) and 400 µM of each nucleotide dATP, dGTP, dCTP, dTTP; 3 mM of $MgCl_2$;plus 28.5 µl of nuclease-free water. All the reactions were performed under the following conditions of amplification: one cycle at 94˚C for 5 min for initial denaturation, followed by 35 cycles at 94˚C for 30 sec, 52˚C for 1 min, 72˚C for 1 min, and a final extension cycle at 72˚C for 5 min. The reaction products were analyzed by electrophoresis in 2.5% agarose gels in Tris-acetate EDTA buffer, stained with ethidium bromide, and visualized under UV light.

DNA Samples from cats positive for CPV-2 were amplified with the following primers ParvoExt1F (5'-ATG AGT GAT GGA GCA GTT CA-3') and ParvoExt3R (5'-AGG TGC TAG TTG AGA TTT TTCATA TAC-3') to obtain 1740 Pb of the VP2 gene. The protocol for PCR amplification with these primers was performed as described by Faz *et al.*[11]

VP2 gene sequencing

In order to verify the CPV-2 antigenic types, amplicons were sequenced; PCR products (25 µL) were submitted to Macrogen USA to be purified having ExoSAP-IT® (Affymetrix) and sequenced using BigDye® v3.1, Life Technologies, Applied Biosystems. all sequences obtained were traduced to amino acid sequences utilizing MEGA 11 software, then they were aligned by means of *ClustalW Software*, and consensus sequences of cats Mexican samples were obtained, these sequences were compared to sequences with accession numbers in *GenBank* (MK671183, FJ011098, KF385391, KY818853, GU362935).

Results

Only 15 (16%) cats with gastroenteritis and 11 (22%) healthy cats were PCR-positive for canine parvovirus (Table 1). In the analysis of the VP2 gene sequences, differentiation between CPV-2 and FPV was achieved using amino acid of the VP2 protein in positions 80, 93, 101, 103, 164, 219, 232, 316, 323, 375, and 564, while amino acid positions in 80, 87, 93, 103, 164, 232, 297, 300, 305, 323, 440, and 564 were different between FPV and CPV-2a; amino acid in position 426 was identified to differentiate CPV-2b, CPV-2c of CPV2a (Table 2).

In healthy cats, CPV-2a not was identified, while in cats with gastroenteritis, ten were infected by CPV-2a, and five more were infected by CPV-2c (Table 1). Samples of the cats infected by CPV-2a were taken in Mexico City and Toluca City states of Mexico; these regions are both geographically close. CPV-2c also infected samples that were obtained from

other states.

Discussion

This report and others previously published[12] demonstrated that CPV-2 variants circulate amongst healthy cats; therefore, they can excrete the virus via their stool.[13] However, some factors favor the lack of identification of CPV-2 in cats. For example, clinical signs of infection by CPV-2 variants in cats are very similar to those produced by FPV; therefore, it is necessary to conduct laboratory tests that are highly sensitive and specific to distinguish between both viruses. In addition, canine parvovirus could be producing signs of disease in cats. However, small animal clinicians commonly do not consider this virus; therefore, infection by CPV-2 has rarely been considered a differential diagnosis to FPV.

We also report that CPV-2a and 2c were identified in healthy cats and in cats with signs of gastroenteritis, and other studies have reported the same.[4] However, there are no existing studies that clearly explain why this occurs. In healthy dogs, a low incidence of canine parvovirus of 1-2% has been reported.[14] In this study, it was observed that variants of CPV-2 could be identified in 22% of the healthy cats evaluated. Some authors indicate that cats are less susceptible to CPV-2 infection than dogs.[15]

Interestingly, we found that in these cats, CPV-2a and 2c were in circulation, compared to sick cats where only CPV-2c was found.[16] In 2001, CPV-2c was reported to be more virulent than CPV-2a and to be dispersed more efficiently amongst cat populations. Recent reports indicated that CPV-2c is the most dispersed antigenic type in America.

Conclusions

In conclusion, this study found that CPV-2 is circulating in the cat population; the antigenic variants infecting Mexican cats are 2c and 2a; therefore, this is the first report about CPV-2a circulating in Mexico. Interestingly, this antigenic type of CPV-2 only was identified in healthy cats, while CPV-2c is most frequent in dogs and cats. Therefore, to better understand why CPV-2a was only found in healthy cats, it is probably necessary to carry out studies with a more significant number of samples obtained from cats with different vaccination conditions, age, and disease degrees.

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Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics approval

The study was approved by the animal ethics committee of the University Center UAEM Amecameca, Autonomous University of the State of Mexico (Acta N° 15, 2021).

Informed consent

Informed consent (verbal) was obtained from the owner of all animals described in this work. No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

Tables

Table 1. Positive samples identification using PCR and sequencing

ID	Origin	Condition	Genotype
24	EDO MEX	H	CPV-2c
25	EDO MEX	H	CPV-2c
26	EDO MEX	H	CPV-2c
34	EDO MEX	H	CPV-2c
38	SLP	H	CPV-2c
39	SLP	H	CPV-2c
41	SLP	H	CPV-2c
42	SLP	H	CPV-2c
43	SLP	H	CPV-2c
44	SLP	H	CPV-2c
45	SLP	H	CPV-2c
77	EDO MEX	A	CPV-2c
78	EDO MEX	A	CPV-2c
80	EDO MEX	A	CPV-2a
81	EDO MEX	A	CPV-2c
84	EDO MEX	A	CPV-2a
85	EDO MEX	A	CPV-2c
86	EDO MEX	A	CPV-2a
91	CDMX	A	CPV-2a
92	CDMX	A	CPV-2a
93	CDMX	A	CPV-2a
94	CDMX	A	CPV-2a
95	CDMX	A	CPV-2a
96	CDMX	A	CPV-2a
97	CDMX	A	CPV-2a
98	CDMX	A	CPV-2c

Table 2. Identification of amino acids that allowed grouping the Mexican cats samples in the different CPV-2 antigenic types (the sequences analyzed in this work were compared with GenBank reference sequences)

VP2 Protein Amino acid number	80	87	93	101	103	164	219	232	297	300	305	316	322	323	375	426	440	564
FPV MK671183	K	M	K	T	V	I	I	V	S	A	D	V	T	D	D	N	T	N
CPV-2 FJ011098	R	M	N	I	A	V	V	I	S	A	D	I	T	N	N	N	T	S
CPV-2a KF385391	R	L	N	T	A	V	I	I	A	G	Y	V	T	N	D	N	A	S
CPV-2a Mex Cat	R	L	N	T	A	V	I	I	A	G	Y	V	T	N	D	N	A	S
CPV-2b KY818853	R	L	N	T	A	V	I	I	A	G	Y	V	S	N	D	D	T	S
CPV-2c GU362935	R	L	N	T	A	V	I	I	A	G	Y	V	T	N	D	E	T	S
CPV-2c Mex Cat	R	L	N	T	A	V	I	I	A	G	Y	V	T	N	D	E	T	S

The amino acids are shown in the code of a letter

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