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ORIGINAL ARTICLE

Origin and genetic diversity of canine parvovirus 2c circulating in Mexico

Mirna Faz[1] · José Simón Martínez[1] · Linda Bautista Gómez[2] · Israel Quijano-Hernández[3] · Raúl Fajardo[1] · Javier Del Ángel-Caraza[3]

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Abstract

Canine parvovirus type 2 (CPV-2) emerged in the late 1970 s as a pathogen that is capable of causing high rates of morbidity and mortality in dogs. Currently, three genetic variants circulate worldwide (CPV 2a, 2b, and 2c); however, epidemiological studies have not been conducted in all countries to identify its variants. The objectives of this work were to determine which genotypes of CPV-2 circulate in Mexico and to identify the genetic relationships between CPV-2 sequences from Mexico and those from other parts of the world. Samples from five geographical regions of Mexico were analysed by PCR for identification of CPV-2. Here, 1638 bp of the VP2 gene were amplified and sequenced from 50 CPV-2-positive samples, and a phylogenetic network was assembled using these 50 sequences and 150 others obtained from GenBank, representing different countries around the world. The network showed that the most common genotype circulating in the geographic zones of Mexico was CPV-2c. In the network, the 50 samples were organised into two clusters: cluster I, derived from a group of samples of European origin, which belong to genotype 2c, and cluster II, derived from samples belonging to genotype 2b from the USA. Our data suggest that the CPV-2 strains circulating in Mexico originated from two possible virus introduction events. In addition, high genetic diversity was observed among the CPV-2c-derived sequences, which correspond exclusively to the presence of Mexican CPV-2c haplotypes.

Introduction

Canine parvovirus type 2 (CPV-2) is one of the most common causes of viral acute enteritis in young dogs [37]. The disease is characterised by vomiting and diarrhoea in affected dogs [33]. The virus is a member of the family *Parvoviridae*, belonging to the genus *Protoparvovirus* and species *Carnivore protoparvovirus 1* [11]. It is a non-enveloped virus with a single-stranded DNA genome that encodes two capsid structural proteins, VP1 and VP2, both of which are required for the assembly and packaging of the viral genome, and NS1 and NS2, which are non-structural proteins that aid in the control of DNA replication, assembly, and regulation of gene expression [1, 43].

CPV-2 emerged as a new virus infecting dogs in the late 1970 s as a host variant of feline panleukopenia virus (FPV), probably through adaptation of an FPV-like parvovirus of wild carnivores. Although there is no absolute evidence, this hypothesis is supported by the active circulation of intermediate viruses between FPV and CPV in wild carnivores and by the inability of FPV to infect dogs [42]. After its first appearance, the original CPV-2 was replaced with a

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✉ José Simón Martínez
jsmartinezc@uaemex.mx

[1] 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, C.P. 50200 Toluca, Estado de México, Mexico

[2] Centro Universitario UAEM Amecameca. Licenciatura en Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Carretera Amecameca Ayapango kilómetro 2.5, C.P. 56900 Amecameca de Juárez, Estado de México, Mexico

[3] Hospital Veterinario de Pequeñas Especies, Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Jesús Carranza No. 203, Col Universidad, Toluca, Estado de México, Mexico

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new variant, Gln-426 (CPV- 2a). The Gln-426 variant further evolved into an Asp-426 variant (CPV- 2b), with both viruses circulating concurrently in dogs worldwide [34]. In 2000, another novel antigenic variant was discovered in Italy [6], characterised by the amino acid (aa) substitution Asp426Glu, and it was subsequently named CPV-2c [15].

CPV- 2c is widespread in European countries (Italy, Germany, Bulgaria and the United Kingdom) [14, 21, 31] and has also been found in North and South America [7, 8, 25, 35, 36], Asia [27, 29, 32] and Africa [3, 41]. Although the first reports seemed to account for the low pathogenicity of CPV-2c, experimental data and field observations have indicated a more severe clinical course and higher mortality rates associated with CPV-2c infection, as well as its ability to infect and cause disease in adult dogs despite frequent vaccination [13, 15–17].

Reports worldwide show that CPV-2 has a substitution rate similar to those of RNA viruses, with values of around 10^{-4} substitutions per site per year [38]. Therefore, the genetic characterisation of new CPV-2 populations is important for the detection of strains with novel biological properties that can provide useful information for CPV-2 epidemiology [12, 30]. Some studies regarding VP2 gene sequences show diversity between the 2c genotype CPVs; consequently, the circulation of these variants in dog populations has allowed the existence of different evolutionary groups or lineages of CPV-2c [2, 24].

The aim of this study was to identify the canine parvovirus genotype circulating in Mexico, using molecular identification of the VP2 gene, and to determine the genetic relationships between Mexican and worldwide isolates.

Materials and methods

Ethical considerations

This study was conducted according to the guidelines of the Experimental Animal Research Mexican Official standard NOM-062-ZOO-1999.

Samples

One hundred fifty stool samples from dogs suspected to be infected with CPV-2 (diarrhoea, vomiting and dehydration) were collected from 13 states of Mexico, corresponding to five geographical areas of the country (Northwest, Northeast, Central, Western and Southeast).

Dogs' stool samples were obtained using sterile rectal swabs, diluted with 500 µL of nuclease-free water, and submitted to our laboratory during 2016; these were tested by PCR following a protocol described previously [20]. Negative samples and patients with a history of vaccination within the previous 15 days were excluded; in total, 50 CPV-2-positive samples were included in the study. Information regarding age, sex, breed, geographical origin, and vaccination status was recorded for all dogs (Table 1).

DNA extraction, PCR, electrophoresis and sequencing

All samples were suspended in nuclease-free water, and 200 µL of the homogenous suspension was used for DNA extraction. The procedure was performed using a QIAamp® DNA Stool DNA Extraction Kit (QIAGEN, Mainz, Germany), following the manufacturer's instructions. All DNA samples were quantified using a Q5000 Quawell spectrophotometer (Quawell Technology, Inc. San Jose, CA, USA).

Each PCR reaction consisted of 1U of GoTaq® Flexi DNA Polymerase (Promega), 5 µL of GoTaq® Flexi Buffer 1X Green, 1.5 mM $MgCl_2$, 10 mM each dNTP, 100 ng of DNA, and 10 µM primers designed in our laboratory using the nucleotide sequences from the VP2 gene (GenBank accession number FJ005196): nt 1-20 for primer ParvoExt1F (5'-ATGAGTGATGGAGCAGTTCA-3') and nt 1,714-1,740 for primer ParvoExt3R (5'-AGGTGCTAGTTGAGATTTTTC ATATAC-3'). The final volume of each reaction was of 50 µL. Amplification conditions were as follows: one initial activation cycle at 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s (denaturation), 50 °C for 45 s (annealing), and 72 °C for 1 min (extension), and one cycle at 72 °C for 5 min (final extension) [20].

All of the amplification products were identified by horizontal electrophoresis in 1% agarose gels stained with 0.5 µg of ethidium bromide per mL and visualised using a UV transilluminator (MiniBIS Pro DNR Bio. Imaging Systems Ltd. Jerusalem, Israel).

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 were 1638 bp in length. The sequences generated in this study were deposited in the GenBank database, with the accession numbers reported in Table 1.

Phylogenetic inferences

The 50 sequences obtained were compared to 150 previously reported sequences available in GenBank from other countries, including Argentina (ARG, $n = 17$), Belgium (BEL, $n = 2$), Brazil (BRA, $n = 4$), China (CHI, $n = 8$), Ecuador (ECU, $n = 12$), France (FRAN, $n = 9$), Germany (GER, $n = 8$), Greece (GRE, $n = 4$), Italy (ITA, $n = 19$), Japan (JAP, $n = 4$), Korea (KOR, $n = 6$), Portugal (PORT, $n = 5$), South Africa (SA, $n = 10$), Spain (SPA, $n = 9$), Taiwan (TAIW, $n = 4$), Thailand (THAI, $n = 4$), Uruguay (URU, $n = 8$), and

Table 1 Characteristics of CPV-2- positive dogs included in this study

ID	GenBank accession number	State	Breed	Sex	Age	Vaccinated
QRO 3	KY818886	Querétaro	Pomeranian	F	3y	+
QRO 6	KY818887	Querétaro	Schnauzer	M	4m	+
QRO 8	KY818888	Querétaro	Bulldog	F	1m	+
GTO 1	KY818859	Guanajuato	Doberman	F	2m	−
GTO 2	KY818860	Guanajuato	Dachshund	F	4m	−
GTO 3	KY818861	Guanajuato	Pit bull	F	1m	−
GTO 4	KY818862	Guanajuato	Crossbreed	M	4m	−
GTO 9	KY818863	Guanajuato	Crossbreed	M	3m	−
NAY 1	KY818879	Nayarit	Schnauzer	F	2m	−
NAY 4	KY818880	Nayarit	Schnauzer	F	2m	−
NAY 6	KY818881	Nayarit	Maltese	M	2m	−
NAY 7	KY818882	Nayarit	Caniche	F	2m	−
NAY 8	KY818883	Nayarit	Chihuahua	M	2y	−
HGO 1	KY818866	Hidalgo	Bulldog	M	3m	+
HGO 2	KY818864	Hidalgo	Basset hound	M	5m	+
HGO 4	KY818867	Hidalgo	Great Dane	M	6m	+
HGO 6	KY818865	Hidalgo	Crossbreed	F	6m	−
HGO 8	KY818868	Hidalgo	Beagle	F	4m	−
SON 1	KY818889	Sonora	Belgian Malinois	M	3m	−
AGC 1	KY818846	Aguascalientes	Labrador retriever	M	2m	−
AGC 2	KY818847	Aguascalientes	Golden retriever	F	6m	−
AGC 4	KY818848	Aguascalientes	Crossbreed	F	4m	−
AGC 5	KY818849	Aguascalientes	Chihuahua	F	2y	−
TAM 1	KY818890	Tamaulipas	Boxer	F	6m	+
TAM 2	KY818891	Tamaulipas	Pit bull	F	5m	+
TAM 3	KY818892	Tamaulipas	German shepherd	F	3m	−
TAM 6	KY818893	Tamaulipas	Boxer	M	2m	−
TAM 7	KY818894	Tamaulipas	Labrador Retriever	M	5m	+
MOR 2	KY818874	Morelos	Belgian Malinois	M	3m	−
MOR 3	KY818875	Morelos	Belgian Malinois	M	3m	−
MOR 4	KY818876	Morelos	Boxer	M	4m	+
MOR 5	KY818877	Morelos	Dogue de Bordeaux	M	4m	−
MOR 6	KY818878	Morelos	Siberian husky	F	3m	+
BCN 6	KY818850	Baja California Norte	Crossbreed	F	3m	−
BCN 7	KY818851	Baja California Norte	Crossbreed	F	4m	−
BCN 8	KY818852	Baja California Norte	Schnauzer	M	5m	−
BCN 9	KY818853	Baja California Norte	Crossbreed	M	2m	−
MICH 1	KY818869	Michoacan	English bull terrier	M	2m	−
MICH 2	KY818870	Michoacan	Chihuahua	M	5m	−
MICH 3	KY818871	Michoacan	Chihuahua	F	5m	−
MICH 4	KY818872	Michoacan	Chihuahua	F	5m	−
MICH 5	KY818873	Michoacan	Crossbreed	M	5m	−
YUC 1	KY818895	Yucatán	Schnauzer	F	3m	+
PUE 1	KY818884	Puebla	Bernese mountain dog	F	6m	−
PUE 2	KY818885	Puebla	Maltese	M	3y	−
MEX71	KY818854	Estado de México	Dachshund	F	8m	+
MEX72	KY818855	Estado de México	Labrador retriever	M	5m	−
MEX79	KY818856	Estado de México	Pomeranian	M	6m	+
MEX82	KY818857	Estado de México	Rottweiler	M	3m	+

Table 1 (continued)

ID	GenBank accession number	State	Breed	Sex	Age	Vaccinated
MEX83	KY818858	Estado de México	Rottweiler	M	3m	+

M, male; F, female; m, months; y, years; +, positive; -, negative

the United States of America (USA, $n = 13$). Four vaccine sequences used by Pfizer, Merial, Fort Dodge and Intervet were also included (Supplementary Table 1).

Sequences were compared using Molecular Evolutionary Genetics Analysis (MEGA) Software v6 with the T92 + G, model (Tamura 3-parameter plus gamma distribution with five rate categories) [40]. To investigate the genetic relationships between all of the sequenced samples, a phylogenetic network was built using the software Network and Network Publisher version 5.0.0.1, using the median-joining algorithm [4].

Analysis of molecular variance (AMOVA) was used to test the correlation between the population genetic structure of the CPV-2 sequences and geographic origin of the samples, vaccination status, age, and sex of the canine host as described by Ohneiser et al. [34]. AMOVA was performed using GenAlEx 6.503. DNA sequences were entered as raw sequences and processed as haploid sequence data to obtain PhiPT values for each analysis. The *P*-values were generated by reference to 1000 random permutations of the input data.

Results

In this study, 50 CPV2-positive samples were sequenced; interestingly, 49 samples were characterized as CPV-2c and only one as CPV-2b, while CPV-2a was not identified. According to the dogs data, only 8% (4/50) of CPV-2-positive dogs were 1 year old or older (1 to 3 years old), and 92% (46/50) were aged between 1 and 8 months. The majority (84%, 42/50) were pure breeds, and approximately half (52%, 26/50) were male.

Regarding vaccination status, 32% (16/50) of the dogs were vaccinated at least twice prior to the CPV-2 infection (Table 1).

Based on alignments of the nucleotide sequences obtained from the 50 dog samples, 71 polymorphic sites were identified. Overall, 64% (32/50) of the samples had different sequences, while 36% (18/50) were organized in seven different haplotypes, which had different repeated sequences: 1 (AGC2 4/50), 2 (AGC5 4/50), 3 (GTO4 2/50) 4 (MOR2 2/50), 5 (MOR4 2/50), 6 (NAY1 2/50) and 7 (TAM1 2/50). Despite the fact that multiple mutations were identified in the analysed sequences, the observed genetic identity was close to 100%.

When Mexican CPV-2 sequences were compared to those reported in other countries, the minimum identity determined was 98.5% with samples from China, Argentina and Taiwan, and the maximum identity was 99.9% with samples from Italy, Germany, Greece and the USA. Finally, the identity observed between Mexican samples and vaccine sequences was 98.4% to 99.7%.

In total, 98% (49/50) of Mexican CPV-2 sequences had the N426E amino acid mutation, indicating that they were CPV-2c isolates. Only 2% (1/50) had the amino acid mutation N426D and were identified as CPV-2b isolates. (This sample is identified as BCN9 in Table 1.) Within the whole sequence, another eight mutations were identified, with the most frequently represented amino acid mutations being T440A and N56D (Table 2).

Regarding the genetic relationships of the Mexican and globally reported sequences, the phylogenetic network showed two clusters where all Mexican samples were contained. Cluster I was composed of a major node, with 20 samples sharing the same genetic sequence, and these viruses originated from Italy, Spain, the USA, France, Portugal, Germany and Greece. Based on this information, 48% (24/50) of the CPV-2 sequences identified in Mexico were organised into 16 unique sequences and four haplotypes: MOR2, MOR4, NAY1, and TAM1, containing two sequences each (Fig. 1).

Cluster II CPV-2c samples identified in Mexico were derived from samples USA5 and USA9, both of which were CPV-2b variants reported in the USA, in 2008 and 2009, respectively. The BCN9 sample was the only one identified as a CPV-2b isolate in our study, and it had USA9 as an ancestor. All samples of this cluster had a common ancestor, ITA10 (CPV-2b), which was reported in Italy in 2005 (Fig. 1). The rest of the network represents CPV-2a, 2b and 2c samples, as reported from different countries from 1993 to 2015, with a predominance of the CPV-2c variant observed in Europe and South America (Fig. 2).

Regarding Mexican samples, an analysis of molecular variance showed a significant correlation between the genetic structure of the CPV-2 sample population and the origin of cluster. In contrast, vaccination status, sex, and the age of the canine host were not significantly correlated with the population genetic structure. With respect to geographic origin of the sample, the genetic structure within the network may be explained only by the 21% genetic variation in the sequences analysed (Table 3).

Table 2 Amino acid variation in the VP2 capsid proteins of the haplotypes identified in Mexican CPV samples

Haplotype name	GenBank accession no.	Amino acid positions							
		56	246	322	352	379	427	440	511
*ITA 1	FJ005210	N	I	T	P	A	D	T	D
BCN 9	KY818853	*	*	S	*	*	*	*	*
QRO 3	KY818886	*	*	*	*	*	*	A	*
QRO 6	KY818887	D	*	*	*	*	*	A	*
GTO 1	KY818859	D	*	*	*	*	*	*	*
GTO 3	KY818861	*	*	*	*	*	*	A	*
GTO 4	KY818862	*	*	*	*	*	*	A	*
NAY 1	KY818879	D	*	*	*	*	*	*	*
NAY 6	KY818881	D	*	*	*	*	*	A	*
NAY 7	KY818882	D	*	*	*	*	*	*	*
NAY 8	KY818883	*	*	*	*	*	*	A	*
HGO 1	KY818866	D	*	*	*	*	*	*	*
HGO 2	KY818864	D	*	*	*	*	*	*	*
HGO 4	KY818867	D	*	*	*	*	*	*	*
HGO 6	KY818865	D	*	*	*	*	*	*	*
HGO 8	KY818868	*	*	*	*	*	*	A	*
SON 1	KY818889	*	*	*	*	*	*	A	*
AGC 1	KY818846	*	*	*	*	*	*	A	*
AGC 2	KY818847	*	*	*	*	*	*	A	*
AGC 5	KY818849	*	*	*	*	*	*	A	*
TAM 1	KY818890	*	*	*	*	*	*	A	*
TAM 2	KY818891	D	*	*	*	*	*	A	*
TAM 3	KY818892	*	*	*	*	*	*	A	*
MOR2	KY818874	D	*	*	*	*	*	*	*
MOR 5	KY818877	D	*	*	*	*	*	*	*
MOR4	KY818876	D	*	*	*	*	*	*	*
BCN 6	KY818850	D	*	S	*	*	*	A	*
BCN 7	KY818851	*	*	S	*	S	*	A	*
BCN 8	KY818852	*	*	S	*	*	*	A	*
MICH 1	KY818869	*	*	*	*	*	*	A	*
MICH 3	KY818871	*	F	*	*	*	*	A	*
MICH 4	KY818872	*	*	*	*	*	*	A	*
YUC 1	KY818895	*	*	*	*	*	*	A	*
PUE 1	KY818884	*	N	*	*	*	*	A	*
PUE 2	KY818885	*	*	*	*	*	*	A	*
MEX71	KY818854	*	*	*	*	*	*	*	V
MEX72	KY818855	*	*	*	*	*	*	A	V
MEX79	KY818856	*	*	*	*	*	N	A	V
MEX82	KY818857	D	*	*	A	*	*	*	*
MEX83	KY818858	D	*	*	*	*	*	*	*

A = alanine, D =aspartic acid, E = glutamic acid, G = glycine, F = phenylalanine, I = isoleucine N = asparagine, P = proline, S = serine, T = threonine, V = valine, R = arginine

*The sequence (FJ005210) was obtained from GenBank and used as reference

Discussion

CPV-2 was identified in the USA in the late 1970 s. In Mexico, the first outbreak of clinical disease was identified in 1980 in a colony of beagles, and was characterised only as CPV-2 [39]. From 1983 to 2000, CPV-2 was quickly replaced by three antigenic variants, CPV-2a, 2b and 2c [6]. These variants have being distributed in approximately 42 countries, and their prevalence is determined by the region and year of collection of the samples [31].

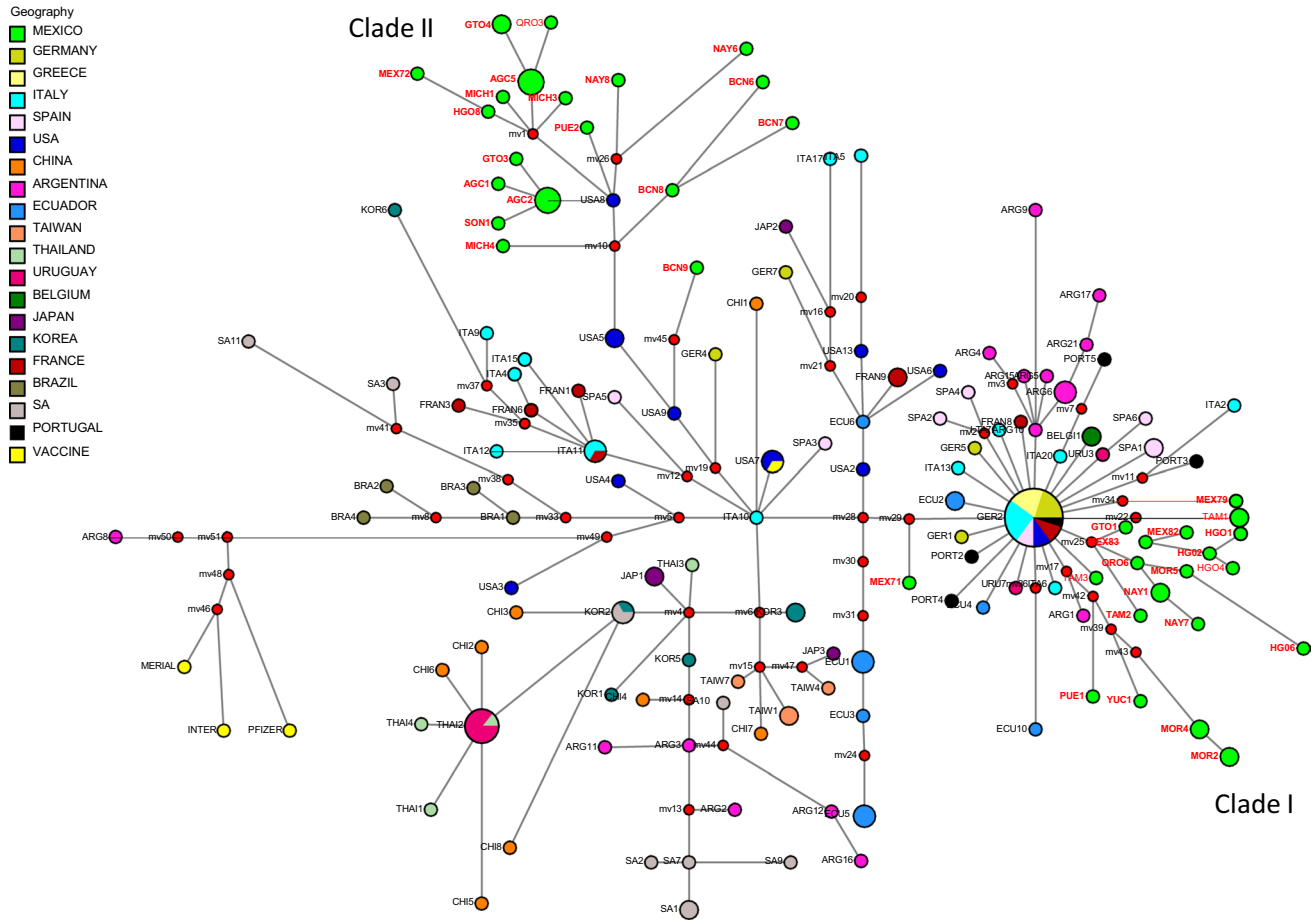

Fig. 1 Haplotype network based on VP2 of canine parvovirus type 2, sequences from samples originating in Mexico (n = 50) and 150 canine parvovirus sequences from 18 countries in GenBank. Nodes are scaled based on the number of representative sequences. The largest node observed within the network contains 20 sequences, while the smaller nodes contain one sequence. Coloured circles indicate the geographic origin of the sample. Inferred nodes are indicated by small closed red circles, as they are not represented among sequences included in the network. The labels show the name of the haplotype containing the following samples: AGC2 (*AGC2, AGC4, GT2, TM7*), AGC5 (*AGC5, MICH2, MICH5, QRO8*), GTO4 (*GTO4, GTO9*), MOR2 (*MOR2, MOR3*), MOR4 (*MOR4, MOR6*), NAY1 (*NAY1, NAY4*) and TAM1 (*TAM1, TAM6*)

Although the presence of CPV-2c has been reported recently in Guadalajara, Mexico, its distribution in others states of the country has not been confirmed [35], and no studies have been conducted to determine the genetic diversity of CPV-2 circulating in affected dog populations in Mexico. In this work, CPV-2 was identified in dogs from different geographical regions of Mexico, and the genetic relationships between these CPV-2 isolates and those reported worldwide were determined.

Overall, 98% of the CPV-2 samples sequenced were identified as CPV-2c isolates. Previously, Pedroza-Roldan and colleagues [35] analyzed samples from the western Mexico region but identified only CPV-2c virus variants. In this work, we confirmed that the most common variant circulating in different geographical areas of Mexico was CPV-2c. Only one sample was identified as CPV-2b. This genotype has not been reported previously in Mexico but has been reported to be circulating in the USA [26]. This sample was obtained from the northeast region of the country, and according to the patient's records, this animal was imported from the USA; therefore, we determined that CPV-2b is not currently circulating frequently in Mexico.

At present, there are no data explaining the phylogenetic origin of CPV-2 circulating in Mexico; our results show that the analysed samples could be divided into two clusters. Cluster I originated from a CPV-2c haplotype that has been identified in different countries in Europe and in the USA. This haplotype was the most common (20/150) among sequences obtained from GenBank, indicating that it is possibly one of the most widely distributed haplotypes worldwide; Grecco et al. [24] concluded that CPV-2c originated in Europe spread throughout South America, and it is therefore likely that the distribution of these strains also occurred in Mexico. They also concluded that the strains

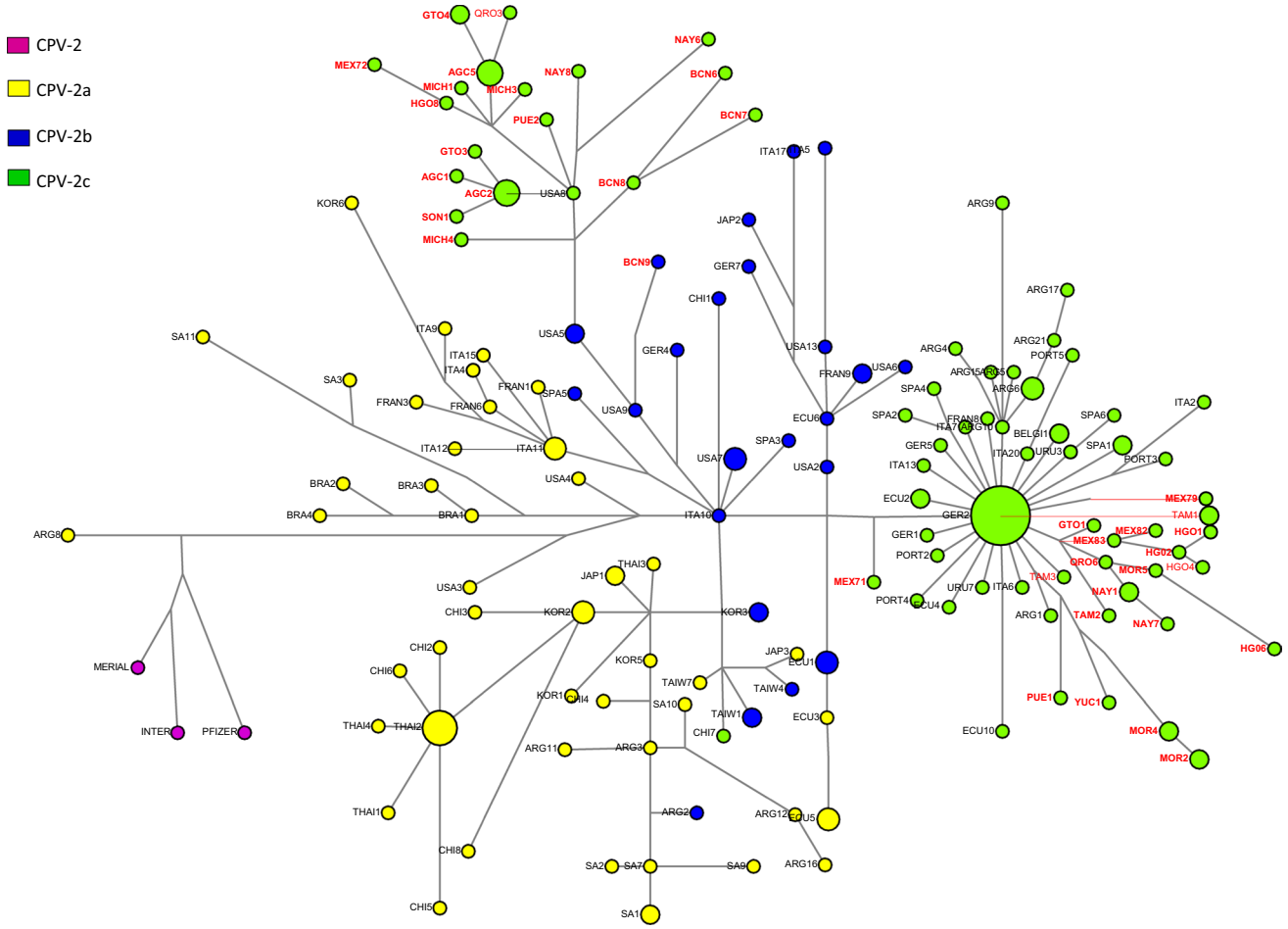

Fig. 2 Haplotype network based on VP2 of canine parvovirus type 2. Nodes are coloured based on the variant of the samples. Mexican haplotype labels are in red

Table 3 AMOVA results for population genetic structure among Mexican canine parvovirus type 2 (CPV-2) sequences

Parameter	n	Variation between populations (%)	Variation within populations (%)	PhiPT	*p*
Geographic origin of the sample: Mexico/world	200 (50/150)	79	21	0.211	0.001
Mexican clusters: I/II in Figure 1	50 (24/26)	59	41	0.412	0.001
Sex of the host: Female/male	50 (24/26)	100	0	-0,018	0,832
Vaccination status: unvaccinated/ vaccinated	50 (34/16)	91	9	0,093	0,004
Age of the host at sampling: Less than one year/one year or older	50 (46/4)	92	8	0,084	0,084

PhiPT: Distribution of variation and differential connectivity among populations

introduced from Europe to America, are currently more common in South American countries. Even in our samples, cluster I indicates that 48% of the analysed samples have a direct CPV-2c-genotype ancestor (Fig. 2). Cluster II, which was observed in 52% of our samples, originated from a CPV-2b genotype reported in Italy. Therefore, it is possible to conclude that the introduction of CPV-2 to Mexico happened in two main events; the first virus introduced had a CPV-2b-genotype virus as an ancestor, and the second one, a CPV-2c-genotype virus.

None of the sequences that we obtained had 100% identity to other previously reported sequences, which could indicate that the CPV-2c variants identified in this work are exclusive to Mexico. This might be due to a high mutation rate, which

is seen in some RNA viruses [38], a similar observation has been made for the virus responsible for canine distemper in Mexico [23], where local genotypes have been identified.

Of the mutations found in the VP2 gene in our samples, 87.6% were synonymous mutations, while 12.4% were non-synonymous. The latter generated different amino acid changes, enabling us to observe that the CPV-2c genotype shows genetic diversity (Table 2). Other researchers have also reported the genetic diversity of CPV-2c [2, 17].

The T440A mutation was found in 50% of our samples. This mutation has been reported frequently in different countries, but the implications of this mutation for CPV-2c biology have not yet been clarified [12, 22, 26, 28]. Other mutations present in our samples, such as A379S and D427N, are associated with amino acids located in the VP2 GH loop, where greater variability has been observed for canine parvovirus [5, 10]. Mutations such as N56D, I246 N/I246F, T322S, D511V and P352A, which had not been reported previously and therefore could be exclusive to Mexican CPV-2c sequences. It is necessary to carry out an in-depth analysis of these samples, in order to determine whether these amino acid substitutions could play a key role in the local viral adaptation.

It has been reported previously that dogs that are naturally infected with any of the three variants, show the same clinical signs [18]; however, some experimental reports and field observations indicate that dogs infected by CPV-2c have a severe clinical profile and high mortality rates [15, 17]. An even higher incidence of infection with CPV-2c has been reported in adult dogs [16], but this is controversial in the opinion of some authors, as they have reported that the clinical profile produced by 2a and 2b variants is similar to that of 2c, with a frequency of 4.5% in adult patients [2, 19]. In this study, only 8% of CPV-2c-infected dogs were adults (1-3 years), all of which presented with clinical signs similar to those identified in puppies (vomiting and haemorrhagic diarrhoea).

Some authors have reported that CPV-2c infection is frequently observed in immunised dogs [7, 16]. Here, we found that 32% of infected dogs had previously been immunised at least once. Some authors discuss the principal causes for the progression of infection, such as the interference of maternally derived antibodies with the virus vaccine [12], while others point to the inability of vaccine strains to provide adequate protection against the field virus [9, 13, 16]. More studies on such topics will be required to determine whether the CPV-2c variant has the capacity to evade the vaccine-stimulated immune response or if the vaccine strains are able to establish protective immunity against it.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Agbandje M, Parrish CR, Rossmann MG (1995) The structure of parvoviruses. Semin Virol 6:299–309
2. Aldaz J, García-Díaz J, Calleros L, Sosa K, Iraola G, Marandino A, Hernández M, Panzera Y, Pérez R (2013) High local genetic diversity of canine parvovirus from Ecuador. Vet Microbiol 166:214–219
3. Amrani N, Desario C, Kadiri A, Cavalli A, Berrada J, Zro K, Sebbar G, Colaianni ML, Parisi A, Elia G, Buonavoglia C, Malik J, Decaro N (2016) Infect Genet Evol 41:201–206
4. Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48
5. Battilani M, Ciulli S, Tisato E, Prosperi S (2002) Genetic analysis of canine parvovirus isolates (CPV-2) from dogs in Italy. Virus Res 83:149–157
6. Buonavoglia C, Martella V, Pratella A, Tempesta M, Cavalli A, Buonavoglia D, Bozzo G, Elia G, Decaro N, Carmichael L (2001) Evidence for evolution of canine parvovirus type 2 in Italy. J Gen Virol 82:3021–3025
7. Calderon MG, Mattion N, Bucafusco D, Fogel F, Remorini P, La Torre J (2009) Molecular characterisation of canine parvovirus strains in Argentina: Detection of the pathogenic variant CPV2c in vaccinated dogs. J Virol Methods 159:141–145
8. Castro TX, Costa EM, Leite JPG, Labarthe NV, Cubel Garcia RCN (2010) Partial VP2 sequencing of canine parvovirus (CPV) strains circulating in the state of Rio de Janeiro, Brazil: Detection of the new variant CPV-2c. Braz J Microbiol 41:1093–1098
9. Cavalli A, Martella V, Desario C, Camero M, Bellacicco AL, De Palo P, Decaro N, Elia G, Buonavoglia C (2008) Evolution of the antigenic relationships among canine parvovirus type 2 variants. Clin Vaccine Immunol 15:534–569
10. Chapman MS, Rossmann MG (1993) Structure, sequence and function correlations among parvoviruses. Virology 194:491–508
11. Cotmore SF, Agbandje-McKenna M, Chiorini JA, Mukha DV, Pintel DJ, Qiu J, Soderlund-Venermo M, Tattersall P, Tijssen P, Gatherer D, Davison AJ (2014) The family Parvoviridae. Arch Virol 159:1239–1247
12. Decaro N, Buonavoglia C (2012) Canine parvovirus-A review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet Microbiol 155:1–12
13. Decaro N, Cirone F, Desario C, Elia G, Lorusso E, Colaianni ML, Martella V, Buonavoglia C (2009) Severe parvovirus in a 12-year-old dog that had been repeatedly vaccinated. Vet Rec 164:593–595
14. Decaro N, Desario C, Addie DD, Martella V, Vieira MJ, Elia G, Zicola A, Davis C, Thompson G, Thiry E, Truyen U, Buonavoglia C (2007) The study molecular epidemiology of canine parvovirus. Eur Emerg Infect Dis 13:1222–1224
15. Decaro N, Desario C, Campolo M, Elia G, Martella V, Ricci D, Lorusso E, Buonavoglia C (2005) Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. J Vet Diagn Invest 17:133–138

16. Decaro N, Desario C, Elia G, Martella V, Mari V, Lavazza A, Nardi M, Buonavoglia C (2008) Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. New Microbiol 31:125–130
17. Decaro N, Desario C, Parisi A, Martella V, Lorusso A, Miccolupo A, Mari V, Colaianni ML, Cavalli A, Di Trani L, Buonavoglia C (2009) Genetic analysis of canine parvovirus type 2c. Virology 385:5–10
18. Decaro N, Martella V, Elia G, Desario C, Campolo M, Lorusso E, Colaianni ML, Lorusso A, Buonavoglia C (2007) Tissue distribution of the antigenic variants of canine parvovirus type 2 in dogs. Vet Microbiol 121:39–44
19. Duque-García Y, Echeverri-Zuluaga M, Trejos-Suarez J, Ruiz-Saenz J (2017) Prevalence and molecular epidemiology of canine parvovirus 2 in diarrheic dogs in Colombia, South America. A possible new CPV-2a is emerging? Vet Microbiol 201:56–61
20. Faz M, Martinez JS, Quijano I, Fajardo R (2017) Reliability of clinical diagnosis and laboratory testing techniques currently used for identification of canine parvovirus enteritis in clinical settings. J Vet Med Sci 79:213–217
21. Filipov C, Desario C, Patouchas O, Eftimov P, Gruichev G, Manov V, Filipov G, Buonavoglia C, Decaro N (2016) A ten-year molecular survey on parvoviruses infecting carnivores in Bulgaria. Transbound Emerg Dis 63:460–464
22. Gallo Calderón M, Wilda M, Boado L, Keller L, Malirat V, Iglesias M, Mattion N, La Torre J (2012) Study of canine parvovirus evolution: comparative analysis of full-length VP2 gene sequences from Argentina and international field strains. Virus Genes 44:32–39
23. Gámiz C, Martella V, Ulloa R, Fajardo R, Quijano-Hernandéz I, Martínez S (2011) Identification of a new genotype of canine distemper virus circulating in America. Vet Res Commun. 35:381–390
24. Grecco S, Iraola G, Decaro N, Alfieri A, Gallo Calderon M, da Silva AP, Name D, Aldaz J, Calleros L, Marandino A, Tomás G, Maya L, Francia L, Panzera Y, Perez R (2018) Inter-and intracontinental migrations and local differentiation have shaped the contemporary epidemiological landscape of canine parvovirus in South America. Virus Evol 4:vey 011
25. Hong C, Decaro N, Desario C, Tanner P, Pardo MC, Sanchez S, Buonavoglia C, Saliki JT (2007) Occurrence of canine parvovirus type 2c in the United States. J Vet Diagn Invest 19:535–539
26. Kapil S, Cooper E, Lamm C, Murray B, Rezabek G, Johnston L, Campbell G, Johnson B (2007) Canine parvovirus types 2c and 2b circulating in North American dogs in 2006 and 2007. J Clin Microbiol 45:4044–4047
27. Kumar M, Nandi S (2010) Molecular typing of canine parvovirus variants by polymerase chain reaction and restriction enzyme analysis. Transbound Emerg Dis 57:458–463
28. Mira F, Dowgier G, Purpari G, Vicari D, Di Bella S, Macaluso G, Gucciardi F, Randazzo V, Decaro N, Guercio A (2018) Molecular typing of a novel canine parvovirus type 2a mutant circulating in Italy. Infect Genet Evol 61:67–73
29. Mira F, Purpari G, Lorusso E, Di Bella S, Gucciardi F, Desario C, Macaluso G, Decaro N, Guercio A (2018) Introduction of Asian canine Parvovirus in Europe through dog importation. Transbound Emerg Dis 65:16–21
30. Miranda C, Parrish CR, Thompson G (2016) Epidemiological evolution of canine parvovirus in the Portuguese domestic dog population. Vet Microbiol 183:37–42
31. Miranda C, Thompson G (2016) Canine parvovirus: the worldwide occurrence of antigenic variants. J Gen Virol 97:2043–2057
32. Nakamura M, Tohya Y, Miyazawa T, Mochizuki M, Phung HT, Nguyen NH, Huynh LM, Nguyen LT, Nguyen PN, Nguyen PV, Nguyen NP, Akashi H (2004) A novel antigenic variant of canine parvovirus from a Vietnamese dog. Arch Virol 149:2261–2269
33. Nandi S, Kumar M (2010) Canine parvovirus: current perspective. Indian J Virol 21:31–44
34. Ohneiser SA, Hills SF, Cave NJ, Passmore D, Dunowska M (2015) Canine parvoviruses in New Zealand form a monophyletic group distinct from the viruses circulating in other parts of the world. Vet Microbiol 178:190–200
35. Pedroza-Roldan C, Paez-Magallan V, Charles-Nino C, Elizondo-Quiroga D, Leonel De Cervantes-Mireles RR, Lopez-Amezcua MA (2015) Genotyping of canine parvovirus in western Mexico. J Vet Diagn Invest 27:107–111
36. Pérez R, Francia L, Romero V, Maya L, López I, Hernández M (2007) First detection of canine parvovirus type 2c in South America. Vet Microbiol 124:147–152
37. Prittie J (2004) Clinical practice review canine parvoviral enteritis: a review of diagnosis, management, and prevention. J Vet Emerg Crit Care 14:167–176
38. Shackelton LA, Parrish CR, Truyen U, Holmes EC (2005) High rate of viral evolution associated with the emergence of carnivore parvovirus. Proc Natl Acad Sci USA 102:379–384
39. Stephano H (1980) Epizootia de enteritis viral canina en Mexico. Posible infeccion por parvovirus. Vet Mex 11:141–148
40. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729
41. Touihri L, Bouzid I, Daoud R, Desario C, El Goulli AF, Decaro N, Ghorbel A, Buonavoglia C, Bahloul C (2009) Molecular characterisation of canine parvovirus-2 variants circulating in Tunisia. Virus Genes 38:249–258
42. Truyen U (2006) Evolution of canine parvovirus—a need for new vaccines? Vet Microbiol 117:9–13
43. Tu M, Liu F, Chen S, Wang M, Cheng A (2015) Role of capsid proteins in parvoviruses infection. Virol J 12:114