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Identification molecular and clinical characterization of CPV-2c in dogs from the state of Mexico.

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Abstract: Canine parvovirus type 2 (CPV-2) is the main etiological agent of viral enteritis in dogs. Actually in literature, CPV-2 has been reported with clinical signs that vary from the classical disease. In this study, we evaluated the clinical signs presented in 50 dogs infected naturally with CPV-2. All the infected dogs were analyzed by PCR and sequenced. Our data indicate that the CPV-2c is the most frequently genovariant in Mexico the 50 dogs belong to the CPV-2c, through the amino acid change 426 Asn-Glu and concerning clinical signs, the presence of either vomiting or enlarged lymph node was observed in all virally infected patients.

Keywords: Canine Parvovirus, Clinical signs, PCR

1. Introduction

CPV-2 is the main etiological agent of viral gastroenteritis in dogs. It is a member of the *Parvoviridae* family, belonging to the *Protoparvovirus* genus and *Protoparvovirus type 1* species. It is a non-enveloped virus with a single stranded DNA genome, which encodes for two capsid proteins, VP1 and VP2, required for the assembly and packaging of the viral genome, as well as for NS1 and NS2 nonstructural proteins, which aid in controlling DNA replication, assembly and regulation of genes expression [11].

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, CPV-2b was identified [10], and in 2001, CPV-2c was detected and reported in Italy. The last variant has also been identified in Asia, Africa and America. The first reports seemed to account for a low pathogenicity of CPV-2c, experimental data and field observations now indicate 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, even if repeatedly vaccinated [4].

The aim of this study was to identify genetically isolated of CPV-2c present in Mexico through the molecular characterization of segment of VP2 gene and characterize clinically infected patients.

2. Results and Discussion

The 100% of dogs belong to the CPV-2c genovariant, through the amino acid change 426 Asn-Glu, 96% were pure bred; 88% of the patients were between one and seven months old, and 12% were older than one year. Concerning their vaccination status, 58% were vaccinated at least once to prevent CPV-2 infection.

Frequency of clinical signs showed by these dogs was as follows: 72% displayed vomiting and diarrhea (catarrhal or hemorrhagic); 14% showed only diarrhea; 7 displayed only vomiting; Leukopenia was observed in 46% of the dogs (Table 1). The analysis of the clinical signs performed through the use

of a chi-squared distribution and the model of logistic regression showed the relationship between PCR and 14 independent variables. The adjusted model equation is:

PCR = -618.314 - 16.6157*VAC - 0.246372*CF + 2.66763*BF + 16.8336*T - 7.93061*AP - 2.14908*LEUK + 1.63731*ED - 53.6041*VOM=0 - 12.9519*DIA=0 - 56.9484*LN=0 - 14.6088*MUC=0 + 57.7868*AP=0 + 17.458*CFT=0 + 38.704*RC=0

As the P-value of the Deviation Analysis is less than 0.05, there is a statistically significant relationship between the variables, with a confidence level of 95.0%. In addition, the P-value for residues is greater than or equal to 0.05, indicating that the model is not significantly worse than the best possible model for these data with a confidence level of 95.0% or greater.

Gastroenteritis caused by CPV-2 is considered one of the main viral diseases that affect dogs. Although clinical signs of canine parvovirus infection may vary, the most common signs reported were: anorexia, depression, lethargy, fever [6,8], mucoid and hemorrhagic diarrhea and leukopenia [1,3,9]; in subclinical cases, some of these signs may or may not be present [5,7]. Clinical variability for this disease has been reported previously [2,5] and some authors have discussed factors, such as age, immune status, exposure route, viral dose, virulence of strains and co-infection with other infectious agents as possible causes [2,7]. Concerning clinical signs, the presence of either vomiting or diarrhea was observed in all virally infected patients, while other clinical signs were not considered relevant to the infection.

3. Materials and Methods

Dogs with clinical enteritis hospitalized in the Veterinary Hospital for Small Animals of the Universidad Autónoma de Estado de México, were screened for this study and selected based on tested positive or negative CPV-2 using PCR. As a result, 50 dogs that tested positive were selected. The 50 dogs were clinically examined by veterinarians to obtain the clinical diagnosis and Information regarding age, breed, vaccination status and clinical outcome of disease were recorded for all dogs.

Dog's stool samples were obtained using rectal swabs, which were suspended in nuclease-free water and 200 µ*l* of the homogenates, and were used for DNA extraction. The procedure was performed

using the 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, U.S.A). 100 *ng* of DNA of each sample were used for PCR reactions with 50 µ*l* of final volume. Previously, a pair of primers was designed in our laboratory to amplify a 275 bp fragment, ParvoInt2FB (5'-TCAAGCAGATGGTGATCCAAG-3') and ParvoInt2CR (5'-GGTACATTATTTAATGCAGTTA-3') located at nucleotides 1,107-1,130 and 1,360-1,382 of the VP2 gene (GenBank accession number FJ0051962c).

PCR reactions were performed using 2 µ*l* of each primer (200 *n*M), 12.5 µ*l* of GoTaq® Green Master Mix (Promega, Madison, WI, U.S.A) containing DNA polymerase, reaction Buffer (pH 8.5) and 400 µM of each nucleotide (dATP, dGTP, Dctp and dTTP); 3 mM of $MgCl_2$. and 28.5 µ*l* of nuclease free water. All reactions were carried out under the following amplification conditions; 1 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.

All the amplification products were identified through horizontal electrophoresis in 2% agarose gels stained with 0.5 *µg*/m*l* of ethidium bromide and visualized with a UV transilluminator. Subsequently 25 µl of PCR product were submitted to Macrogen USA to be purified by ExoSAP-IT® (Affymetrix) and sequenced by BigDye® v3.1 Life Technologies, Applied Biosystems. All sequences were aligned using the Software MEGA 6.0 (Tamura et al., 2007). The analysis of the clinical signs was performed through the use of a chi-squared distribution and the model of logistic regression.

4. Conclusion

Our data indicate that the CPV-2c is the most frequently genovariant in our Mexican dogs and concerning clinical signs, the presence of vomiting and enlarged lymph node was observed in all virally infected patients, while other clinical signs were not considered relevant to the infection.

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Conflicts of Interest

The authors declare no conflict of interest.

References and Notes

1. Calderon, M. G.; Mattion, N.; Bucafusco, D.; Fogel, F.; Remorini, P. and La Torre, J. 2009. Gallo, M.; Nora, M.; Bucafusco, D.; Fogel, F.; Remolino, P. and La Torre, J. 2009. Molecular characterization of canine parvovirus strains in Argentina: detection of the pathogenic variant CPV2c in vaccinated dogs. *J. Virol. Methods* 159: 141–145.
2. Decaro, N. and Buonavoglia, C. 2012. Canine parvovirus-A review of epidemiological and diagnostic aspects with emphasis on type 2c. *Vet. Microbiol.*155:1–12.
3. Decaro, N.; Desario, C.; Elia, G.; Martella, V.; Mari, V.; Lavazza, A.; Nardi, M. and Buonavoglia, C. 2008. Evidence for immunization failure in vaccinated adult dogs infected with canine parvovirus type 2c. *New Microbiol.* 31:125-130.
4. Decaro, N.; Desario, C.; Parisi, A.; Martella, V.; Lorusso A.; Miccolupo, A.; Mari, V.; Loredana, M.; Cavalli, A.; Di Trani.; L and Buonavoglia, C. 2009. Genetic analysis of canine parvovirus type 2c. *Virology* 385: 5-10.
5. Decaro, N.; Elia, G.; Martella, V.; Desario, C.; Campolo, M.; Di Trani, L.; Tarsitano, E.; Tempesta, M. and Buonavoglia, C. 2005. A real-time PCR assay for rapid detection and quantitation of canine parvovirus type 2 in the feces of dogs. *Vet. Microbiol.* 105:19-28.
6. Goddard, A. and Leisewitz, A. 2010. Canine parvovirus. *Vet. Clin. North Am. Small Anim. Pract.* 40: 1041-1053.
7. Lamm, C. and Rezabek, G. 2008. Parvovirus infection in domestic companion animals. *Vet. Clin. North Am. Small Anim. Pract*. 38: 837-850.
8. Prittie, J. 2004. Canine parvoviral enteritis: a review of diagnosis, management, and prevention. *J. Vet. Emerg. Crit. Care* 14: 167-176.
9. Schoeman, J.; Goddard, A. and Leisewitz, A. 2013. Biomarkers in canine parvovirus enteritis. *New. Zeal. Vet. J*. 6: 217-222. **21**

10. Truyen, U. 2006. Evolution of canine parvovirus-A a need for new vaccines? *Vet.Microbiol.*117: 9-13. **23**
11. Tu, M.; Liu, F.; Chen, S.; Wang, M and Cheng, A. 2015.Mengyu, T.; Fei, L.; Shun, C.; Mingshu, W. and Anchung, C. 2015. Role of capsid protein in parvoviruses infection. *Virol. J.* 12: 114. **24**

Table 1. Clinical signs and characteristics presented by the patients (50 dogs tested positive to CPV-2 PCR).

N°	Age	Sex	Vaccines	CF	RC	CFT	BF	AP	Mucous	T°	LN	Diarrhea	Vomiting	Leukocytes $6x10^3/\mu L$
1	10 m	F	0	100	-	> 2 Sec	28	Pain	Pink	38.5	N	-	+	29.9
2	1 y	F	4	120	-	> 2 Sec	30	Pain	Pink	38	↑	+	+	17.2
3	3 m	M	1	140	-	> 2 Sec	22	Pain	Pink	38.4	↑	+	+	8
4	5 y	M	4	120	-	> 2 Sec	40	Pain	Pink	39.3	N	+	-	19
5	7 m	M	3	112	-	> 2 Sec	45	Pain	Pink	38.8	↑	+	-	10.3
6	4 m	F	0	150	-	> 2 Sec	18	Pain	Pink	38.6	↑	+	+	8.1
7	10 m	F	3	100	-	> 2 Sec	44	Pain	Pink	39.2	N	+	-	9
8	4 m	M	1	120	-	> 2 Sec	33	Pain	Pink	39.3	↑	+	+	5.3
9	3 m	M	2	50	-	> 2 Sec	30	Pain	Pale	38.2	↑	+	+	9
10	2 m	M	0	120	-	> 2 Sec	20	Pain	Pale	37.1	↑	+	+	3
11	1 y	M	0	150	+	> 2 Sec	22	Pain	Pale	34.5	↑	-	+	18
12	1 m	M	0	140	-	> 2 Sec	36	Pain	Pink	38.4	N	+	+	13.4
13	5 m	F	2	160	-	> 2 Sec	44	Pain	Pink	39.6	↑	+	+	10.2
14	5 m	M	2	120	-	> 3 Sec	20	Pain	Pink	40	N	+	+	3
15	1 m	F	0	100	+	> 2 Sec	30	Pain	Pink	38.7	N	+	+	11.1
16	5 m	F	1	124	-	> 3 Sec	64	Pain	Pink	40.8	N	+	+	4
17	5 m	M	0	92	-	> 2 Sec	20	Pain	Pale	38.8	↑	+	+	3.1
18	3 m	F	0	136	-	> 3 Sec	20	Pain	Pale	38.8	N	+	+	6.1
19	1 y	M	2	140	-	> 3 Sec	20	Pain	Pale	39.6	N	+	+	1.2
20	5 m	F	3	110	-	> 3 Sec	20	Pain	Pale	38.7	↑	-	+	8.2
21	2 m	F	2	160	-	> 2 Sec	20	Pain	Pink	38	↑	+	+	4.1
22	2 m	M	2	180	-	> 3 Sec	30	Pain	Pink	37.9	↑	-	+	4.2
23	4 m	M	0	204	-	> 3 Sec	24	Pain	Pink	38.8	↑	+	+	2.2
24	3 m	M	1	200	-	> 3 Sec	30	Pain	Pink	39.5	N	+	+	2.9
25	2 m	F	1	110	-	> 3 Sec	20	Pain	Pink	37.1	↑	+	-	23.6
26	3 m	F	0	138	-	> 2 Sec	22	Pain	Pale	38.1	↑	+	+	2.7
27	4 m	M	1	176	-	> 2 Sec	28	Pain	Pale	37.8	↑	+	+	0.95
28	3 m	M	0	132	-	> 2 Sec	56	Pain	Pinks	39.9	↑	+	+	1.2
29	3 m	M	0	120	-	> 2 Sec	30	Pain	Pinks	38.6	N	+	+	3.4
30	2 y	M	0	170	-	> 3 Sec	28	Pain	Pinks	38.3	↑	+	+	3.4
31	7 m	F	3	146	-	> 2 Sec	28	Pain	Pinks	38.6	↑	+	-	3.2
32	4 m	M	0	140	-	> 2 Sec	20	Pain	Pale	36.4	N	-	+	3.7
33	2 años	F	4	146	-	> 2 Sec	26	Pain	Pink	37.8	↑	+	+	24
34	4 m	M	0	126	-	> 2 Sec	20	Pain	Pink	40.3	↑	+	+	1.2
35	5 m	M	0	160	-	> 2 Sec	28	Pain	Pink	37.7	N	+	+	2
36	5 m	M	1	160	-	> 3 Sec	24	Pain	Pale	37.7	↑	+	+	3.3
37	3 m	M	3	140	-	> 2 Sec	20	Pain	Pale	38.4	↑	+	+	9
38	4 m	M	0	140	-	> 2 Sec	24	Pain	Pink	39.6	↑	+	+	8.2
39	3m	M	3	120	-	> 3 Sec	26	Pain	Pale	38.8	↑	+	+	14.9
40	4 m	F	2	100	-	> 2 Sec	24	Pain	Pink	39.2	N	+	+	6.2
41	5 m	M	0	127	-	> 2 Sec	28	Pain	Pink	38.1	N	+	+	11.9
42	4 m	F	0	216	-	> 2 Sec	28	Pain	Pale	38.1	↑	+	+	16.1
43	2 m	M	2	140	-	> 2 Sec	32	Pain	Pale	38.7	N	+	-	14.2
44	3 m	M	0	100	-	> 2 Sec	46	Pain	Pale	38.5	↑	+	+	3.5

45	4 m	M	1	140	-	> 3 Sec	40	Pain	Pale	39.5	↑	+	+	5.8
46	4 m	F	3	128	-	> 2 Sec	18	Pain	Pale	37.6	↑	+	+	1.3
47	3 m	M	1	142	-	> 2 Sec	25	Pain	Pink	39.8	↑	+	-	6.5
48	3 m	F	0	180	-	> 2 Sec	24	Pain	Pale	37.4	↑	-	+	4.2
49	4 m	F	3	180	-	> 2 Sec	38	Pain	Pink	40.1	↑	-	+	7.9
50	7 m	M	4	187	-	> 2 Sec	26	Pain	Pale	38.9	↑	+	+	5.3

(m) months; (y) years; (F) Female; (M) Male; (CF) Cardiac Frequency; (RC) Reflex cough; (+) Positive or present during the study; (-) Negative or absent during the study; (CFT) Capillary filling time; (BF) Breathing frequency; (AP) Abdominal palpation; (T°) Temperature °C; (LN) Lymph node; (N)Normal; (↑) Increased in size.