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MICROBIOLOGY

Genetic characterization and predominance of the new CPV-2a variant in clinical cases of canine parvovirus in the western region of Rio Grande do Sul, Brazil

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ABSTRACT: Canine parvovirus 2 (CPV-2) is an important causative agent of segmental enteritis in young dogs and has globally distributed variants and subtypes. Viral mutations can alter the pathogenesis and clinical signs, making identifying the samples circulating in a given region relevant. This study described the epidemiological and clinical findings and the molecular characterization of CPV-2 samples circulating in the canine population of Uruguaiana, Rio Grande do Sul (RS), Brazil. We analyzed 27 cases with a complete clinical history and at least one confirmatory etiologic diagnosis. In addition to clinical and epidemiological data, whole blood samples or tissues were tested by PCR for viral DNA detection. Amplified products were sequenced and analyzed, and phylogeny was generated with reference sequences. The disease was diagnosed especially in the summer months, and the most common clinical findings were diarrhea, anorexia, listlessness, and vomiting. Infection was predominant in young (< 6 months) unvaccinated or partially immunized dogs, with mortality exceeding 93%. It was possible to identify 15 CPV-2 samples, four of which were CPV-2a and 11 were new CPV-2a. It can be concluded that canine parvovirus is a disease with high mortality rates, with young unvaccinated dogs being more susceptible, with a predominance of the new CPV-2a variant in the western region of Rio Grande do Sul.
Key words: gastroenteric syndrome, puppy, diarrhea, dogs, parvovirus.

Caracterização genética e predominância da variante do novo CPV-2a em casos clínicos de parvovirose canina na região oeste do Rio Grande do Sul, Brasil

RESUMO: O parvovírus canino 2 (CPV-2) é um importante agente etiológico de enterite segmentar em cães jovens e apresenta variantes e subtipos distribuídos mundialmente. As mutações virais podem alterar a patogenia e os sinais clínicos, o que torna relevante identificar as amostras que circulam em determinada região. O objetivo do trabalho foi descrever os achados epidemiológicos, clínicos e a caracterização molecular das amostras de CPV-2 circulantes na população canina do município de Uruguaiana, Rio Grande do Sul (RS), Brasil. Foram analisados 27 casos clínicos que possuíam histórico clínico completo e pelo menos um diagnóstico etiológico confirmatório. Além dos dados clínicos e epidemiológicos, amostras de sangue total, suabes retais ou tecidos foram testados por PCR para detecção do DNA viral. Os produtos amplificados foram sequenciados, analisados e a filogenia gerada com sequências de referência. A doença foi diagnosticada especialmente nos meses de verão e os achados clínicos mais comuns foram diarreia, anorexia, apatia e vômito. A infecção foi predominante em cães jovens (< 6 meses) não vacinados ou parcialmente imunizados, com mortalidade superior a 93%. Foi possível identificar quinze amostras de CPV-2, sendo quatro CPV-2a e onze new CPV-2a. Pode-se concluir que a parvovirose canina é uma enfermidade com altos índices de mortalidade, sendo os cães jovens não vacinados mais susceptíveis, havendo predominância da variante new CPV-2a na região oeste do RS.
Palavras-chave: síndrome gastroentérica, filhote, diarreia, cães, parvovírus.

INTRODUCTION

Gastrointestinal diseases have relevance in the medical practice of canines, especially young ones, and viral enteritis is among the most common causes of infectious diarrhea (KANTERE et al., 2021). Canine parvovirosis is an enteric infection with varying degrees of severity caused by the *Protoparvovirus carnivoran* 1 (Canine parvovirus type 2 - CPV-2) which can be hemorrhagic with a high mortality rate (DECARO & BUONAVOGLIA, 2012, WALKER et al., 2022). The clinical infection manifests mainly in young dogs up to 12 months of age, with no sex or breed predisposition observed (CASTRO et al., 2007; DECARO & BUONAVOGLIA, 2012; OLIVEIRA et al., 2018).

CPV-2 has a tropism for cells with high mitosis rates, such as intestinal crypts and lymphoid organ cells (DECARO & BUONAVOGLIA, 2012). Clinically, the infection is characterized by anorexia, lethargy, apathy, vomiting, leukopenia, and diarrhea, which can range from pasty to hemorrhagic. Fatal acute fulminant myocarditis can occur in animals up to eight weeks of age (DECARO et al., 2005;

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SOUTO et al., 2018; LAMM & GRANT, 2008; KANTERE et al., 2021).

Belonging to the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Protoparvovirus*, CPV-2 is a non-enveloped virus, measuring approximately 26 nm (WALKER et al., 2022). The ssDNA genome, composed of two open reading frames (ORFs), with ORF1 encoding for non-structural proteins (NS1 and NS2), and ORF2 responsible for encoding the structural proteins (VP1, VP2, and VP3). CPV-2 originated from feline panleukopenia virus (FPLV) in the 1970s, where mutations in the capsid protein VP2 enabled the virus to use the canine transferrin receptor and establish infection in a new host (PARRISH, 1991; TRUYEN, 2006; HOELZER & PARRISH, 2010). Despite having the DNA genome, the mutation rate of CPV-2 can be considered high. This characteristic has enabled the emergence of different variants over time (DECARO et al., 2005; KWAN et al., 2021). Between the years 1978 and 1981, mutations at positions 426 (asparagine to aspartic acid) and position 555 (isoleucine to valine) in amino acids of the VP2 gave rise to the variants CPV-2a and CPV-2b (PARRISH, 1991). In 2001, a new mutation was identified at residue 426, which gave rise to variant CPV-2c (asparagine to glutamic acid) (BUONAVOGLIA et al., 2001). In addition, CPV-2-like subtypes, new CPV-2a and new CPV-2b have been identified; however, aspects of pathogenesis and immune reactivity remain unclear (CALDERON et al., 2009; PINTOS et al., 2011; OLIVEIRA et al., 2019; MIRANDA & GERTRUDE 2016; MIRA et al., 2019). New CPV2a/2b variants contain a Ser297Ala mutation in addition to the mutation at amino acid 426 of VP2, which is their molecular signature (DECARO & BUONAVOGLIA, 2012; WU et al., 2015; OLIVEIRA et al., 2019).

Classification of CPV-2 into variants and subtypes is based on the presence of amino acid residues at positions 87, 139, 267, 297, 300, 324, 347, 440, and 555 of the viral capsid VP2 protein (BUONAVOGLIA et al., 2001; OLIVEIRA et al., 2019; SAGAZIO et al., 1998; WU et al., 2015). Mutations are associated with modifications of epitopes recognized by B lymphocytes and regions responsible for virus-cell interaction. These mutations may confer advantages in viral replication and affect pathogenesis (DECARO & BUONAVOGLIA, 2012). Thus, the identification of CPV-2 samples and clinical characterization is essential for determining these aspects of infection and evaluating control, prevention, and treatment measures. Therefore, this study described the clinical and epidemiological findings and the molecular identification of CPV-2 samples circulating in the canine population on the western border of Rio Grande do Sul, Brazil.

MATERIALS AND METHODS

Clinical case selection: we selected 27 clinically diagnosed cases of gastroenteric syndrome seen at a private veterinary hospital or submitted for necropsy in Uruguaiana, RS, Brazil. Only cases with clinical and epidemiological descriptions, accompanied by at least one laboratory diagnosis, were considered for inclusion in the study.

Clinical cases and samples: the animals were subjected to a complete clinical examination, complementary exams (hemogram), and blood and/or stool collection for immunochromatography and/or PCR. During clinical care, epidemiological data was collected (age, breed, and vaccination history). Small intestine samples were also collected from dogs necropsied after confirmation or clinical suspicion of parvovirus or with intestinal lesions suggestive of the disease on macroscopic examination. The Ethics Committee on Animal Use approved all clinical evaluation procedures and sample collection at Universidade Federal do Pampa (CEUA registration 016/2021).

Anatomopathology: fragments of the small intestine were collected during necropsy, fixed in 10% formalin, and processed according to routine. Tissue segments were stained with hematoxylin and eosin and evaluated by light microscopy to confirm the histopathological diagnosis.

Immunochromatography: rectal swab samples were tested for antigen by immunochromatography test, according to the manufacturer's instructions (Alere®).

DNA extraction: samples of whole blood (500 µL), or intestinal fragments (+/- 1 gram) were used for extraction of total DNA by the phenol-chloroform method (SAMBROOK & RUSSEL, 2001). Gut samples were processed previously DNA extraction and clarified to remove contaminants, as described by Oliveira et al. (2018). The final product was resuspended in 32 µL of TE pH 8.0 (Tris-EDTA), quantified, and stored at -80 °C.

Amplification of CPV-2 VP2: total DNA from blood samples (n = 12) and intestine fragments (n = 15) were subjected to partial amplification of the VP2 gene using primers 555for and 555rev, as described by Buonavoglia et al. (2001). The final amplification result (583 bp) was visualized in a 1.5% agarose gel, stained by Gel Red® (Sigma-Aldrich), under ultraviolet light, after electrophoresis.

Sample identification and phylogenetic analysis: the PCR products were purified and sequenced (ACTGene Análises Moleculares Ltda, Alvorada, RS, Brazil) in duplicate. The sequences obtained were submitted to the Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST/) for comparison with the sequences already deposited in GenBank, edited by the BioEdit Sequence Alignment Editor Software suite, version 7.0.5.3 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) to obtain the consensus sequence. All the sequences (from this study and from GenBank) were translated and the program Molecular Evolutionary Genetics Analysis (MEGA - version X 20) was used for phylogenetic analysis with bootstrap values of 1000. The program JModel Test was used to determine the best evolutionary model of the sequences used and determined the statistical model K80+I as indicated. Identification of CPV-2 variants was performed based on amino acid identification at residue 426 of the VP2 protein.

RESULTS

The data presented described the occurrence and characteristics of canine parvovirus in the western region of Rio Grande do Sul, Brazil. All cases reported were subject to clinical routine or sent for necropsy. Diarrhea was the clinical sign present in 100% of cases, followed by anorexia (66.6%), apathy (44.4%), and vomiting (40.7%). Stool consistency ranged from mushy diarrhea (7.4%), liquid diarrhea (33.3%), or bloody diarrhea (59.3%). WBC data were available for 20 animals, and in ten cases, leukopenia could be identified (data not shown). (Table 1).

Table 1 - Epidemiological and clinical characteristics, analyzed matrix, diagnostic assay and CPV-2 variant identified in clinical cases in Uruguaiana, the western border region of Rio Grande do Sul, Brazil.

ID (LV)	-------Breed-------	----------Clinical signs----------				Matrix	--Diagnostic assay--	CPV-2 variant
		Apathy	Anorexia	Vomiting	Diarrhea			
07/17	Mongrel		+	+	+	SI[1]	PCR	CPV-2a
09/17	Schnauzer[2]	+	+	+	+	SI	Histopathology, PCR	new CPV-2a
16/17	Schnauzer[2]		+	+	+	SI	Histopathology	
21/17	Mongrel	+			+	SI	PCR	CPV-2a
15/18	Spitz Alemão	+		+	+	SI	Histopathology, PCR	new CPV-2a
08/20	Shih Tzu		+		+	SI	IMC[3], PCR	CPV-2a
01/21-03	Pitbull Terrier	+			+	Blood	IMC, PCR	new CPV-2a
01/21-04	Shih Tzu				+	Blood	IMC, PCR	
01/21-05	Yorkshire				+	Blood	IMC, PCR	new CPV-2a
01/21-06	Border Collie	+		+	+	Blood	IMC, PCR	new CPV-2a
30/20-01	German Shepherd		+	+	+	Blood	IMC, PCR	new CPV-2a
30/20-02	Mongrel		+		+	Blood	IMC	
30/20-03	Mongrel		+		+	Blood	IMC, PCR	
30/20-04	Mongrel		+		+	SI	IMC, PCR	new CPV-2a
12/21-01	Pinscher	+	+	+	+	Blood	IMC, PCR	new CPV-2a
12/21-02	Mongrel	+	+		+	Blood	IMC	
13/21-01	Yorkshire	+	+		+	SI	IMC	
13/21-02	French Bulldog	+	+		+	SI	IMC, PCR	
22/21-01	Mongrel		+		+	SI	IMC	
22/21-02	Shih Tzu	+	+		+	SI	IMC	
22/21-03	Mongrel		+		+	SI	IMC, PCR	
30/21-01	Mongrel	+	+		+	Blood	IMC, PCR	CPV-2a
31/21	Mongrel				+	Blood	IMC, PCR	new CPV-2a
32/21	Shih Tzu			+	+	Blood	IMC, PCR	new CPV-2a
33/21-01	Shih Tzu[2]	+	+	+	+	SI	IMC	
33/21-02	Shih Tzu[2]			+	+	SI	IMC	
33/21-03	Shih Tzu[2]		+	+	+	SI	IMC, PCR	

1 - Small intestine;
2 - Same litter;
3 - IMC: immunochromatography tested on rectal swab samples.

The viral etiology was simultaneously confirmed through immunochromatography testing with rectal swab samples from 22 cases. The occurrence of cases was distributed between September and April, suggesting probable seasonality (Figure 1A). No cases were diagnosed between May and August, characterized by fall and winter (Figure 1A). All affected animals were less than one year old, and 77.7% were less than three months old (Figure 1B). In two situations (LV 09/17 and 16/17 and LV 33/21-01, 33/21-02 and 33/21-03), litters were affected. Dogs of different breeds and Mongrels (non-defined breed) were affected. Vaccination history indicated that only 7.4% had a complete vaccination schedule, and all others (92.6%) were non vaccinated or had incomplete vaccination schedules (Figure 1C). It was not possible to obtain reliable information about living with other dogs in the residence or access to the street. Among the 27 selected cases, only two animals survived.

DNA was extracted from blood (n = 12), small intestine (n = 15) and submitted to PCR. The CPV-2 genome was detected in 20 samples, which were sent for sequencing in duplicate. In the end, 15 sequences (~ 495 bp) of sufficient quality were obtained for the analyses (Figure 2). Comparison with

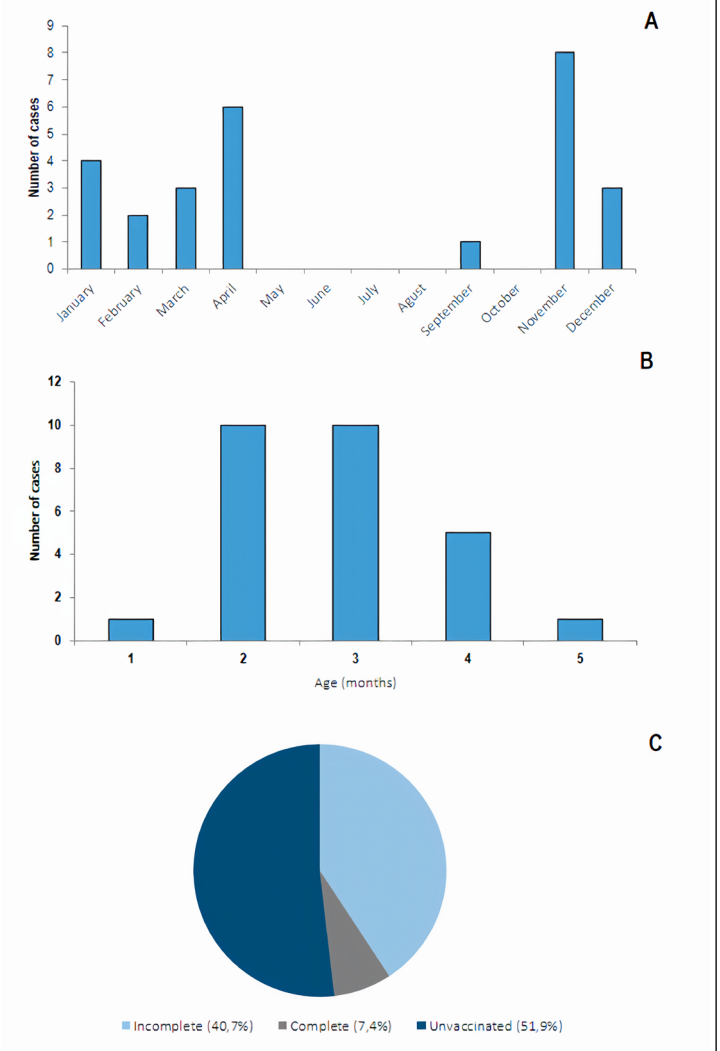

Figure 1 - Temporal distribution (A), age (B), and vaccination status (C) of dogs diagnosed with canine parvovirus in the municipality of Uruguaiana, Rio Grande do Sul, Brazil.

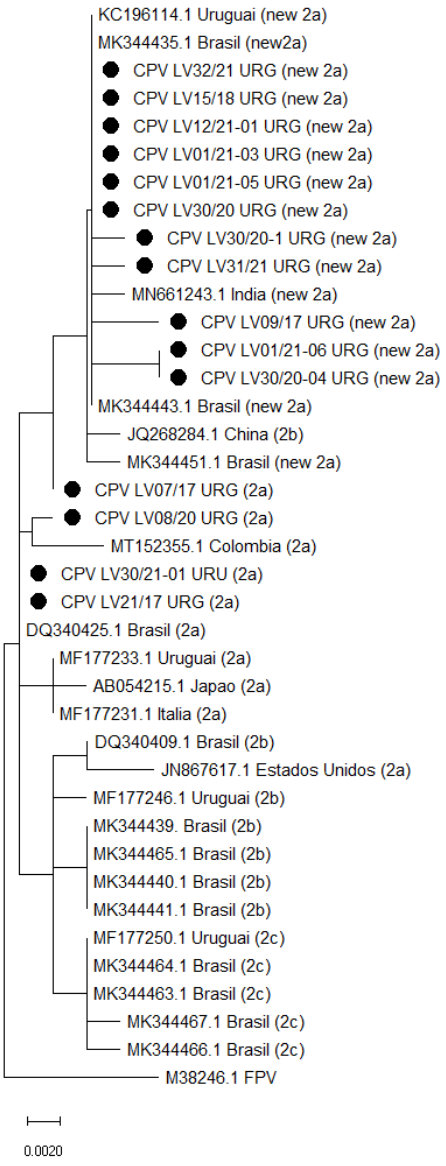

Figure 2 - Phylogenetic analysis of the samples identified in clinical cases of canine parvovirus disease in Uruguaiana, Rio Grande do Sul, Brazil. Phylogenetic analysis of the sequenced samples was performed with the program Molecular Evolutionary Genetics Analysis (MEGA) version 10 with values of 1000 bootstraps. The program JModel Test was used to determine the best evolutionary model of the sequences used and determined the statistical model K80+I as indicated.

sequences deposited in GenBank revealed similarity between 98% to 100% with CPV-2 samples. The analysis of the amino acids encoded by the obtained sequences revealed that 4/15 have asparagine at position 426 and isoleucine 555, classifying them as variant CPV-2a.

Additionally, for 11 samples, the presence of a threonine or alanine at position 440 was also identified, characterizing them as sub-variant new CPV-2a. The phylogeny based on the amino-acidic sequence revealed that the samples identified as new CPV-2a (n = 11) group with the same virus sub-variants of Brazilian, Indian, and Uruguayan origin. Four samples classified as CPV-2a grouped in a cluster with classical, Brazilian, Japanese, Italian, and Uruguayan sequences of the same variant (Figure 2).

DISCUSSION

The presented data refer to the clinical presentation of parvovirosis in a city in western Rio Grande do Sul, Brazil (Table 1, figures 1 and 2). The described cases illustrate how the agent manifests itself in the studied canine population and which viral variants are circulating. The clinical signs commonly showed were diarrhea, apathy, anorexia, and vomiting. These signs are characteristic of canine parvoviruses; however, diarrhea was present in all cases (OLIVEIRA et al., 2018; DECARO et al., 2005; OLIVEIRA et al., 2009; TUTEJA et al., 2022).

The study showed that clinical, epidemiological, and virological characteristics among cases corroborate with other studies (CALDERON et al., 2011; DECARO & BUONAVOGLIA, 2012; KANTERE et al., 2021; LAMM & GRANT, 2008). The affected animal breeds in this study should be analyzed with caution, as it may represent the most prevalent breeds in the region, without defining that those breeds are more sensitive to the disease.

High mortality is characteristic of canine parvovirosis, and several studies indicate varying degrees of lethality (CALDERON et al., 2011; DECARO & BUONAVOGLIA, 2012; NANDI et al., 2010; SOUTO et al., 2018). Young animals under the age of 6 months are the most affected, probably due to the association of factors such as reduced maternal immunity, high intestinal mitosis rate, and presence of co-infections that can aggravate the infection (DECARO et al., 2005; DECARO et al., 2020; SOUTO et al., 2018). Dogs older than two years have already developed natural immunity from exposure to the agent or have higher vaccination coverage (DECARO et al., 2020).

The 95% mortality of the animals found in the present study may result from the association between the timing of clinical care and the intensity of clinical signs. Combining these factors worsens the

clinical picture and makes therapeutic intervention ineffective. Still, the signs of CPV-2 infection may be complicated due to secondary infections caused by a canine coronavirus, bacteria, helminths, and intestinal protozoa (CASTRO et al., 2007; OLIVEIRA et al., 2009). In the present study, in at least one case, an intense infection by *Dipylidium* sp. was diagnosed (ID # 12). In newborn dogs, myocarditis is another lesion that can occur; however, it is underreported, and this may be associated with the lack of clinical and histopathological diagnosis (SOUTO et al., 2018).

The reduction of clinical signs and severity of the clinical picture may occur through the combination of actions such as the vaccination of pregnant bitches and no exposure of puppies to contaminated environments before completing the vaccination scheme (DECARO et al., 2020). Another critical aspect of preventing deaths is clinical intervention soon after the appearance of the first signs. Immunochromatography tests enable fast, accurate diagnosis and are widely used in clinical routine, besides having a high assertiveness rate (DECARO & BUONAVOGLIA, 2012; DESARIO et al., 2005). In addition, early etiologic diagnosis is fundamental for the correct orientation of therapeutic procedures and animal isolation (DECARO & BUONAVOGLIA, 2012).

All cases were identified between September and April, with a higher concentration in the summer months (December - March) and no record of occurrence in the winter months. Although our results suggest seasonality, this is not a characteristic of canine parvoviruses that can be diagnosed throughout the year (CASTRO et al., 2007; QI et al., 2020). However, the concentration of diagnosis in the period of more significant heat may be associated with increased activities of dogs and guardians in outdoor spaces. This may contribute to dogs staying for long periods in possibly contaminated environments (KANTERE et al., 2021; QI et al., 2020).

Vaccination for canine parvovirus is the main form of disease control, reducing cases in young animals and the severity of clinical signs (DECARO et al., 2020). Dogs with an unvaccinated or incomplete vaccination schedule were the most affected (96.3%). Most commercial vaccines have the original CPV-2a and/or CPV-2b strains in their composition. Some authors advocate updating vaccines and including other variants, such as CPV-2c. This fact would be associated with updated information on the molecular epidemiology of CPV variants (MITTAL et al., 2014; SPIBEY et al., 2008). However, this assumption seems to be debatable, as several studies describe the predominance of variants that would be protected by vaccines (DECARO et al., 2020; GIRALDO-RAMIREZ et al., 2020; SAGAZIO et al., 1998; WILSON et al., 2014). Despite the differences between the vaccine composition and the viral strain circulating in the canine population, it is observed that canine parvoviruses are primarily diagnosed in dogs without a vaccine or with an incomplete vaccination scheme (DECARO et al., 2020; MITTAL et al., 2014). This hypothesis can be observed in the present study, where only 7.4% of the affected dogs were fully vaccinated (three doses) (Figure 1C). In addition to vaccine quality, aspects such as storage and application, the dog's immunological status, and time after the scheme's completion should be observed because they influence vaccine immunity (CALDERON et al., 2009; CASTRO et al., 2011; DECARO et al., 2020). Vaccination of dogs in contaminated or hygiene-deficient environments should be avoided (MICHAEL et al., 2017).

Interestingly, in the present study, the new CPV-2a variant was the predominant one (11/15) (Figure 2 and table 2). More accurate identification of other sub-variants would only be possible with complete sequencing of VP2 as proposed by (OLIVEIRA et al., 2018). Several studies propose the replacement of variants 2a and 2b for the 2c variant (OLIVEIRA et al., 2019; SPIBEY et al., 2008). In the present study, we exclusively identified the CPV-2a and new CPV-2a variants; nevertheless, it is not possible to rule out the circulation of the 2b or 2c variants in the canine population (GIRALDO-RAMIREZ et al., 2020). The evolution of CPV-2 is mainly related to the mutation rate of the agent, which can be considered high for a DNA virus (DECARO & BUONAVOGLIA, 2012; KWAN et al., 2021; SAGAZIO et al., 1998). Other factors, such as vaccination coverage and interactions between populations, should also contribute to the emergence of new variants. All variants identified maintain the same pathogenicity and virulence characteristics in young, unimmunized dogs. The only exception would be CPV-2c, which could infect vaccinated ones, but this observation needs further substantiation (OLIVEIRA et al., 2018).

The emergence of one variant replacing the others is an event that occurs in specific populations independently and simultaneously. This event results from several factors (mutation, vaccination coverage, and population interaction) and varies in intensity according to the region and its characteristics. This would explain the uneven distribution of variants and

Table 2 - Amino acid profile of the carboxy-terminal region of VP2 found in CPV-2a, new CPV-2a, CPV-2b, and CPV-2c strains and samples identified in clinical cases in the city of Uruguaiana, Rio Grande do Sul, Brazil.

Variant	-------Sample-------	----------Amino acid----------			Accession number
		426	440	555	
CPV-2a	CPV-LZ1	Asn	Thr	Val	JQ268283.1
	LV 07/17	Asn	Thr	Val	OR546404
	LV 21/17	Asn	Thr	Val	OR546403
	LV 08/20	Asn	Thr	Val	OR546406
	LV 30/21-01	Asn	Thr	Val	OR546402
new CPV-2a	CPV M306	Asn	Ala	Val	KC196114.1
	LV 09/17	Asn	Ala	Val	OR546407
	LV 15/18	Asn	Ala	Val	OR546414
	LV 01/21-03	Asn	Ala	Val	OR546411
	LV 01/21-05	Asn	Ala	Val	OR546410
	LV 01/21-06	Asn	Ala	Val	OR546409
	LV 30/20-01	Asn	Ala	Val	OR546401
	LV 30/20-04	Asn	Ala	Val	OR546413
	LV 12/21-01	Asn	Ala	Val	OR546412
	LV 31/21	Asn	Ala	Val	OR546408
	LV 32/21	Asn	Ala	Val	OR546415
CPV-2b	CPV/23	Asp	Thr	Val	MW653251.1
CPV-2C	CPV 5 MGL	Glu	Thr	Val	MH660909.1

the emergence of new variants in specific populations (CALDERON et al., 2011; BUONAVOGLIA et al., 2001). Still, it is essential to note that the complete analysis of VP2 could reveal the occurrence of new sub-variants (OLIVEIRA et al., 2019). Therefore, the analysis of variants circulating in certain regions should consider the populations' vaccination coverage and breeding characteristics.

Phylogenetic analysis showed that the samples sequenced and identified as CPV-2a clustered with reference sequences and other viruses identified in Brazil and Japan. New CPV-2a samples clustered with other similar samples of Brazilian and Uruguayan origin. The reduced number of samples and the short evaluation period may not represent the total circulation of virus variants in the region, and it is impossible to rule out the circulation of other variants. In some countries such as Australia, India, Hungary, South Korea, Greece, and China, CPV-2a and new CPV-2a variants are believed to be predominant (CHINCHKAR et al., 2006; DEMETER et al., 2010; MEERS et al., 2007; NDIANA et al., 2021; ZHANG et al., 2010). In Brazil, the prevalence of the variants varies according to the time and region of the study. The predominant variants in the 1980s and 1990s were 2b and 2a; respectively (PEREIRA et al., 2000). In the early 2000s, variant 2a was predominant, and subsequently, 2c was widely identified (CASTRO et al., 2010; PINTO et al., 2012).

CONCLUSION

The occurrence of canine parvovirus is an important cause of gastroenteric syndrome in animals younger than six months of age, unvaccinated, and results in high mortality. Diarrhea is the most frequent sign, followed by anorexia, listlessness, and vomiting. The CPV-2a and new CPV-2a variants were the only ones identified, with the latter being the most frequent.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

Conceptualization: MCSB, CKT and PFF. Data acquisition: BCL, JCSJ, MET and BLA. Design of methodology and data analysis: BCL, JCSJ and MCSB. BCL and JCSJ prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

The Ethics Committee on Animal Use approved all clinical evaluation and sample collection procedures at Universidade Federal do Pampa (CEUA registration 016/2021).

REFERENCES

BUONAVOGLIA, C. et al. Evidence for evolution of canine parvovirus type 2 in Italy. **Journal of General Virology**, v.82, p.3021-3025, 2001. Available from: <https://www.microbiologyresearch.org/content/journal/jgv/10.1099/0022-1317-82-12-3021#tab2>. Accessed: Feb. 20, 2022. doi: 10.1099/0022-1317-82-12-3021.

CALDERON, M. G. et al. Molecular characterization of canine parvovirus strains in Argentina: detection of the pathogenic variant CPV2c in vaccinated dogs. **Journal of Virological Methods**, v.159, p.141-145, 2009. Available from: <https://www.sciencedirect.com/science/article/pii/S0166093409001165>. Accessed: Mar. 20, 2022. doi: 10.1016/j.jviromet.2009.03.013.

CALDERON, M. G. et al. Evolution of canine parvovirus in Argentina between years 2003 and 2010: CPV2c has become the predominant variant affecting the domestic dog population. **Virus Research**, v.157, p.106-110, 2011. Available from: <https://www.microbiologyresearch.org/content/journal/jgv/10.1099/0022-1317-82-12-3021#tab2>. Accessed: Feb. 20, 2022. doi: 10.1016/j.virusres.2011.02.015.

CASTRO, T. X. et al. Partial VP2 sequencing of canine parvovirus (CPV) strains circulating in the state of Rio de Janeiro, Brazil: detection of the new variant CPV-2c. **Brazilian Journal of Microbiology**, v.41, p.1093–1098, 2010. Available from: <https://www.scielo.br/j/bjm/a/BkWBNTxQsqz8jdnvShZNr3x/?lang=en>. Accessed: Mar. 20, 2022. doi: 10.1590/S1517-838220100004000031.

CASTRO, T. X. et al. Monitoring of canine parvovirus (CPV) strains detected in vaccinated puppies in Brazil. **Research in Veterinary Science**, v.90, p.336-340, 2011. Available from: <https://www.sciencedirect.com/science/article/pii/S0034528810002122>. Accessed: Feb. 20, 2022. doi: 10.1016/j.rvsc.2010.06.005.

CASTRO, T. X. et al. Clinical and epidemiological aspects of canine parvovirus (CPV) enteritis in the State of Rio de Janeiro: 1995 - 2004. **Arquivo Brasileiro de Medicina Veterinária e Zootecnia**, v.59, p.333–339, 2007. Available from: <https://www.scielo.br/j/abmvz/a/b8fsXkdrrMgTqqMXZ6FMMxb/?lang=en>. Accessed: Oct. 10, 2020. doi: 10.1590/S0102-09352007000200010.

CHINCHKAR, S. R. et al. Analysis of VP2 gene sequences of canine parvovirus isolates in India. **Archives of Virology**, v.151, p.1881-1887, 2006. Available from: <https://link.springer.com/article/10.1007/s00705-006-0753-8>. Accessed: Mar. 05, 2022. doi: 10.1007/s00705-006-0753-8.

DECARO, N.; BUONAVOGLIA, C. Canine parvovirus--a review of epidemiological and diagnostic aspects, with emphasis on type 2c. **Veterinary Microbiology**, v.155, p.1-12, 2012 Available from: <https://www.sciencedirect.com/science/article/pii/S0378113511005013>. Accessed: Feb. 20, 2022. doi: 10.1016/j.vetmic.2011.09.007.

DECARO, N. et al. Canine parvovirus vaccination and immunization failures: are we far from disease eradication? **Veterinary Microbiology**, v.247, p.108760, 2020. Available from: <https://www.sciencedirect.com/science/article/pii/S0378113520305654>. Accessed: Aug. 03, 2022. doi: 10.1016/j.vetmic.2020.108760.

DECARO, N. et al. Clinical and virological findings in pups naturally infected by canine parvovirus type 2 Glu-426 mutant. **Journal of Veterinary Diagnostic Investigation**, v.17, p.133-138, 2005. Available from: <https://journals.sagepub.com/doi/10.1177/104063870501700206>. Accessed: Feb. 20, 2022. doi: 10.1177/104063870501700206.

DEMETER, Z. et al. Misleading results of the MboII-based identification of type 2a canine parvovirus strains from Hungary reacting as type 2c strains. **Virus Genes**, v.41, p.37-42, 2010. Available from: <https://link.springer.com/article/10.1007/s11262-010-0478-3>. Accessed: Dec. 30, 2022. doi: 10.1007/s11262-010-0478-3.

DESARIO, C. et al. Canine parvovirus infection: which diagnostic test for virus? **Journal of Virological Methods**, v.126, p.179-185, 2005. Available from: <https://www.sciencedirect.com/science/article/pii/S0166093405000650>. Accessed: Jul. 02, 2020. doi: 10.1016/j.jviromet.2005.02.006.

GIRALDO-RAMIREZ, S. et al. Phylogenetic, evolutionary, and structural analysis of canine parvovirus (CPV-2) antigenic variants circulating in Colombia. **Viruses**, v.12, p.30, 2020. Available from: <https://www.mdpi.com/1999-4915/12/5/500>. Accessed: Nov. 07, 2020. doi: 10.3390/v12050500.

HOELZER, K.; PARRISH, C. R. The emergence of parvoviruses of carnivores. **Veterinary Research**, v.41, p.39-52, 2010. Available from: <https://www.vetres.org/10.1051/vetres/2010011>. Accessed: Mar. 01, 2022. doi: 10.1051/vetres/2010011.

KANTERE, M. et al. Risk and environmental factors associated with the presence of canine parvovirus type 2 in diarrheic dogs from Thessaly, **Central Greece. Pathogens**, v.10, p.590, 2021. Available from: <https://www.mdpi.com/2076-0817/10/5/590>. Accessed: Feb. 20, 2022 doi.org/10.3390/pathogens10050590.

KWAN, E. et al. Analysis of canine parvoviruses circulating in Australia reveals predominance of variant 2b and identifies feline parvovirus-like mutations in the capsid proteins. **Transboundary and Emerging Diseases**, v.68, p.656-666, 2021. Available from: <https://onlinelibrary.wiley.com/doi/full/10.1111/tbed.13727>. Accessed: Mar. 08, 2023. doi: 10.1111/tbed.13727.

LAMM, C. G.; GRANT, B. R. Parvovirus infection in domestic companion animals. **Veterinary Clinics of North America**, v.38, p.837-850, 2008. Available from: <https://www.sciencedirect.com/science/article/pii/S0195561608000818>. Accessed: Jun. 20, 2022. doi: 10.1016/j.cvsm.2008.03.008.

MEERS, J. et al. Genetic analysis of canine parvovirus from dogs in Australia. **Australian Veterinary Journal**, v.85, p.392–396, 2007. Available from: <https://onlinelibrary.wiley.com/doi/10.1111/j.1751-0813.2007.00206.x>. Accessed: Jun. 11, 2021. doi: 10.1111/J.1751-0813.2007.00206.X.

MICHAEL, J. D. et al. Small animal vaccination: a practical guide for vets in the UK. **World Small Animal Veterinary Association**, v.39, p.110-118, 2017. Available from: <https://wsava.org/wp-content/uploads/2020/01/Small-animal-vaccination-a-practical-guide-for-vets-in-the-UK-Michael-Day.pdf>. Accessed: Feb. 20, 2022.

MIRA, F. et al. Spreading of canine parvovirus type 2c mutants of Asian origin in southern Italy. **Transboundary and Emerging Diseases**, v.66, p.2297–2304, 2019. Available from: <https://onlinelibrary.wiley.com/doi/10.1111/tbed.13283>. Accessed: Sept. 05, 2021. doi: 10.1111/tbed.13283.

MIRANDA, C.; GERTRUDE, T. Canine parvovirus: the worldwide occurrence of antigenic variants. **Journal of General Virology**, v.97 p.2043-2057 2016. Available from: <https://www.microbiologyresearch.org/content/journal/jgv/10.1099/jgv.0.000540#tab2>. Accessed: Oct. 06, 2021. doi: 10.1099/jgv.0.000540.

MITTAL, M. et al. Molecular typing of canine parvovirus strains circulating from 2008 to 2012 in an organized kennel in India reveals the possibility of vaccination failure. **Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases**, v.23, p.1-6, 2014. Available from: <https://www.sciencedirect.com/science/article/pii/S1567134814000185>. Accessed: Feb. 20, 2022. doi: 10.1016/j.meegid.2014.01.015.

NANDI, S. et al. Occurrence of canine parvovirus type 2c in the dogs with haemorrhagic enteritis in India. **Research in Veterinary Science**, v.88, p.169-171, 2010. Available from: <https://www.sciencedirect.com/science/article/pii/S0034528809001428>. Accessed: Feb. 20, 2022. doi: 10.1016/j.rvsc.2009.05.018.

NDIANA, L. A. et al. Molecular characterization of canine parvovirus from domestic dogs in Nigeria: introduction and spread of a CPV-2c mutant and replacement of older CPV-2a by the "new CPV-2a" strain. **Virus Disease**, v.32, p.361-368, 2021. Available from: <https://link.springer.com/article/10.1007/s13337-021-00689-0>. Accessed: Jan. 02, 2022. doi: 10.1007/s13337-021-00689-0.

OLIVEIRA, E. C. et al. Immunohistochemical analysis of dogs infected naturally by canine parvovirus. **Pesquisa Veterinária Brasileira**, v.29, p.131–136, 2009. Available from: <https://www.scielo.br/j/pvb/a/B75Gz4jX34bXhKsfKxVLQWC/abstract/?lang=pt>. Accessed: Feb. 20, 2022. doi: 10.1590/S0100-736X2009000200008.

OLIVEIRA, P. S. B. et al. Epidemiological, clinical and pathological features of canine parvovirus 2c infection in dogs from southern Brazil. **Pesquisa Veterinária Brasileira**, v.38, p.113–118, 2018. Available from: <https://www.scielo.br/j/pvb/a/T6fZzkvVmzfVsJKnbtSKjXG/>. Accessed: Mar. 15, 2022. doi: 10.1590/1678-5150-PVB-5122.

OLIVEIRA, P. S. B. et al. New variants of canine parvovirus in dogs in southern Brazil. **Archives of Virology**, v.164, p.1361-1369, 2019. Available from: <https://link.springer.com/article/10.1007/s00705-019-04198-w>. Accessed: Feb. 20, 2022. doi: 10.1007/s00705-019-04198-w.

PARRISH, C. R. Mapping specific functions in the capsid structure of canine parvovirus and feline panleukopenia virus using infectious plasmid clones. **Virology**, v.183, p.195-205, 1991. Available from: <https://www.sciencedirect.com/science/article/pii/004268229190132U>. Accessed: Dec. 06, 2023. doi: 10.1016/0042-6822(91)90132-u.

PEREIRA, C. A. et al. Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. **Veterinary Microbiology**, v.75, p.127-133, 2000. Available from: <https://www.sciencedirect.com/science/article/pii/S0378113500002145>. Accessed: Feb. 20, 2022. doi: 10.1016/s0378-1135(00)00214-5.

PINTO, L. D. et al. Typing of canine parvovirus strains circulating in Brazil between 2008 and 2010. **Virus Research**, v.165, p.29-33, 2012. Available from: <https://www.sciencedirect.com/science/article/pii/S0168170212000020>. Accessed: Oct. 20, 2020. doi: 10.1016/j.virusres.2012.01.001.

PINTOS, A. B. L. et al. Isolation and characterization of canine parvovirus type 2c circulating in Uruguay. **Ciência Rural**, v.41, p.1436–1440, 2011. Available from: <https://www.scielo.br/j/cr/a/zhzxNCKTZBtjSKsPNbJqGTL/?lang=en>. Accessed: Feb. 20, 2022. doi: 10.1590/S0103-84782011005000098.

QI, S. et al. A Mini-review on the epidemiology of canine parvovirus in China. **Frontiers in Veterinary Science**, v.7, p.1-10, 2020. Available from: <https://www.frontiersin.org/articles/10.3389/fvets.2020.00005/full>. Accessed: Sept. 20, 2022. doi:.3389/FVETS.2020.00005/BIBTEX.

SAGAZIO, P. et al. Antigenic characterization of canine parvovirus strains isolated in Italy. **Journal of Virological Methods**, v.73, p.197-200, 1998. Available from: <https://www.sciencedirect.com/science/article/pii/S016609349800055X?via%3Dihub>. Accessed: Jan. 15, 2022. doi: 10.1016/S0166-0934(98)00055-X.

SAMBROOK, J.; RUSSEL, D. W. Rapid isolation DNA. **Molecular cloning, a laboratory manual**. Edition 02, Cold Spring Harbor Laboratory Press, 1989.

SOUTO, E. P. F. Outbreak of parvoviral myocarditis in puppies in Brazil. **Pesquisa Veterinária Brasileira**, v.38, p.94–98, 2018. Available from: <https://www.scielo.br/j/pvb/a/F7PvyMPNYFQftTP5DvgV6YB/?lang=pt>. Accessed: Nov. 20, 2022. doi: 10.1590/1678-5150-PVB-4787.

SPIBEY, N. et al. Canine parvovirus type 2 vaccine protects against virulent challenge with type 2c virus. **Veterinary Microbiology**, v.128, p.48–55, 2008. Available from: <https://www.sciencedirect.com/science/article/pii/S0378113507004737?via%3Dihub>. Accessed: Feb. 20, 2022. doi: 10.1016/j.vetmic.2007.09.015.

TRUYEN, U. Evolution of canine parvovirus - a need for new vaccines? **Veterinary Microbiology**, v.117, p.9-13, 2006. Available from: <https://www.sciencedirect.com/science/article/pii/S0378113506001374>. Accessed: Mar. 09, 2023. doi:10.1016/j.vetmic.2006.04.003.

TUTEJA, D. et al. Canine parvovirology – a brief updated review on structural biology, occurrence, pathogenesis, clinical diagnosis, treatment and prevention. **Microbiology and Infectious Diseases**, v.82, p.e101765, 2022. Available from: <https://www.sciencedirect.com/science/article/pii/S0147957122000224>. Accessed: Aug. 20, 2022. doi: 10.1016/j.cimid.2022.101765.

WALKER, P. J. et al. Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses. **Archives of Virology**, v.167, p.2429–2440, 2002. Available from: <https://link.springer.com/article/10.1007/s00705-022-05516-5>. Accessed: Oct. 01, 2023. doi: 10.1007/s00705-022-05516-5.

WILSON, S. et al. Vaccination of dogs with canine parvovirus type 2b (CPV-2b) induces neutralizing antibody responses

to CPV-2a and CPV-2c. **Vaccine**, v.32, p.5420–5424, 2014. Available from: <https://www.sciencedirect.com/science/article/pii/S0264410X14010846>. Accessed: Feb. 20, 2022. doi: 10.1016/J.VACCINE.2014.07.102.

WU, J. et al. Molecular epidemiological and phylogenetic analyses of canine parvovirus in domestic dogs and cats in Beijing, 2010–2013. **Journal of Veterinary Medical Science**, v.77, p.2010–2013, 2015. Available from: <https://www.jstage.jst.go.jp/article/jvms/77/10/77_14-0665/_article>. Accessed: Oct. 03, 2023. doi: 10.1292/jvms.14-0665.

ZHANG, R. et al. Phylogenetic analysis of the VP2 gene of canine parvoviruses circulating in China. **Virus Genes**, v.40, p.397–402, 2010. Available from: <https://link.springer.com/article/10.1007/s11262-010-0466-7>. Accessed: Dec. 20, 2022. doi: 10.1007/s11262-010-0466-7.