Heliyon

ELSEVIER

CrossMark

Circulation of canine parvovirus among dogs living in human-wildlife interface in the Atlantic forest biome, Brazil

Flávia V. Vieira [a], **Daniel J. Hoffmann** [a], **Carolina U.F. Fabri** [a], **Katia D.S. Bresciani** [a], **Roberto Gameiro** [a], **Eduardo F. Flores** [b], **Tereza C. Cardoso** [a,*]

[a] *São Paulo State University (UNESP), School of Veterinary Medicine Araçatuba, Laboratory of Animal Virology and Cell Culture Araçatuba, São Paulo, Brazil,16050-680*

[b] *Setor de Virologia, Departamento de Medicina Veterinária Preventiva, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil. 97105-900*

* Corresponding author at: Departamento de Apoio, Produção e Saúde Animal, Curso de Medicina Veterinária, Rua Clóvis Pestana, 793, 16050–680 Araçatuba, SP, Brazil.

E-mail address: tcardoso@fmva.unesp.br (T.C. Cardoso).

Abstract

Despite of the role of domestic dogs as reservoirs for threatening viral diseases for wild carnivores, few studies have focused to identify circulation of viruses among dogs living in human/wildlife interfaces. To identify canine parvovirus (CPV) types circulating in dogs living in an Atlantic forest biome, faecal samples (n = 100) were collected at the same period (one week) corresponding to each of four areas, during 2014 to 2016 and corresponded to 100 different individuals. CPV was isolated in cell culture from 67 out 100 (67%) samples from healthy dogs. Cytopathic effects were characterized by total or partial cell culture lysis. Genome sequences of CPV-2a (10%), CPV-2b (7%) and CPV-2c (50%) were concomitantly detected by PCR and nucleotide sequencing. The current study addresses the importance of monitoring CPV circulation among dogs presenting potential contact with wildlife species.

Keywords: Veterinary science, Microbiology, Virology, Infectious disease

1. Introduction

Since its initial identification in the late 1970's, canine parvovirus (CPV) has been evolving, generating new genetic and antigenic variants worldwide. Since its emergence, CPV has remained a common and important cause of morbidity and mortality in young dogs worldwide (Decaro et al., 2009; Decaro et al., 2011; Miranda and Thompson, 2016). The CPV evolution has given rise to three genetic/ antigenic variants, CPV-2a, CPV-2b and CPV-2c (Vieira et al., 2008; Decaro and Buonavoglia, 2012). Molecular and epidemiological data indicate that the newest type, CPV-2c, is becoming prevalent in several geographic areas and is often associated with severe disease in adult dogs, including those with complete vaccination record (Decaro and Buonavoglia, 2012). In Brazil, the CPV-2c variant has already been described, especially in southern Brazil (Costa et al., 2005; Castro et al., 2010; Oliveira et al., 2017).

The furthermost important diseases transmitted from dogs to wild carnivores, e.g. rabies, canine distemper and parvovirosis, are named " The Big Three" due to the strong impact over the health of wild species (Curi et al., 2016). Thus, infectious diseases driven-mortality is one of the major causes of population decline and, in some situations, even extinction of wild mammal carnivores worldwide (Curi et al., 2016). Although evidence on the role of domestic dogs as reservoirs of viral diseases for wild carnivores is becoming a global pattern, few such reports are described in South America (Acosta-Jamett et al., 2015; Curi et al., 2016). In this respect, a pioneer study has recently described the prevalence and risk factors for viral exposure in rural dogs around protected areas of Atlantic forest (Curi et al., 2016). For this purpose, virus isolation, PCR detection and sequencing analysis of isolated CPV were performed in 100 faecal samples collected from dogs living in a rural area belonging to Atlantic forest biome

2. Material and methods

2.1. Samples and virus isolation

Faecal samples were collected per rectum from apparently healthy dogs and stored at -86 °C prior to virus isolation. The studied region is localized 21°12′32″S 50°25′58″W and was divided into 4 different areas (50 Km^2). The collection was performed at the same period (one week) corresponding to each area, during 2014 to 2016 and corresponded to 100 different individuals. This study was approved by the ethical committee (CEEA) of Universidade Estadual Paulista "Júlio de Mesquita Filho". The study was performed under all applicable institutional guidelines for the care and ethical use of animals were followed as recommended by Animal Brazilian Experimentation Committee protocol number 2015/09754. Approximately 2 g of faeces was homogenized in 1 volume of sterile phosphate-buffered solution (PBS), clarified by centrifugation at 2,500 x g for 10 min. The

supernatant was filtered through a 0.75 μm filter (Millipore[TM]) and treated with amphotericin and penicillin (concentrated at 100X; Sigma-Aldrich[®], St. Louis, MO, USA). AF-72 cells (ATCC, CRL 1542) were cultured in MEM (Sigma-Aldrich[®]) supplemented with antimycotic/antibiotic 1X solution, 10% foetal calf serum (Sigma-Aldrich[®]), 2 mM L-glutamine (Sigma-Aldrich[®]), and non-essential amino acids (100x, Invitrogen[®], Life Technologies, Carlsbad, CA, USA). Cultures were incubated at 37 °C in 5% CO_2 with 95% humidity. After AF-72 cells reached 80% confluence, 1 ml of faecal preparation was added to 4.7×10^5 cells/ml and the culture supernatant was submitted to three blind passages at 5-day intervals. Inoculated and control cells were monitored under phase-contrast using an Olympus IX–70 microscope for production of cytopathic effect (CPE) (Olympus[®], Tokyo, Japan). Approximately 10 fields were analysed in each condition, and photographs were taken at 200 x magnification by using cell Sens[TM] software (Olympus[®]). CPV VR-2016[TM] (ATCC), Cornell strain, which is present in all Brazilian vaccines, were used as controls. Mock-infected AF-72 cells were used as negative controls nd cells inoculated with CPV VR-2016[TM], strain Cornell were used as positive controls. Other enteric viruses, e.g., canine adenoviruses 1 and 2 (CAV-1, CAV-2) and canine distemper virus (CDV), were not detected in the examined samples by molecular analysis (data not shown).

2.2. Molecular analysis

For detection of the CPV genome, total DNA from infected/uninfected AF-72 cells was extracted using DNAzol[TM] according to the manufacturer's instructions (Invitrogen[®]). An average of 100 ng of genomic DNA was used for PCR as described previously. Polymerase chain reaction (PCR) was performed to amplify a fragment of 583 base pairs (bp) of the VP2 gene, a region surrounding position 426, using primers described previously (Decaro et al., 2009; Mohan Raj et al., 2010). CPV amplicons were purified using the NucleoSpin Extract II kit and sequenced with an ABI PRISM 3100 Genetic Analyser (Applied Biosystems[TM]) using the BigDye Terminator v. 3.1Cycle Sequencing Kit (Applied Biosystems[TM]). Sequences were aligned using BioEdit Sequence Alignment Editor V.7.0.9.0 (Tamura et al., 2007). The evolutionary history was inferred using the Neighbour-Joining method (Saitou and Nei, 1987). The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree (Hall, 1999). All positions containing gaps and missing data were eliminated from the dataset. Phylogenetic analyses were conducted in MEGA4 (Tamura et al., 2007). A phylogenetic tree was constructed based on amino acid alignments using BLAST analysis (www.blast.ncib.nlm.nih.gov/Blast.cgi), and sequences generated for CPV were submitted to GenBank and assigned.
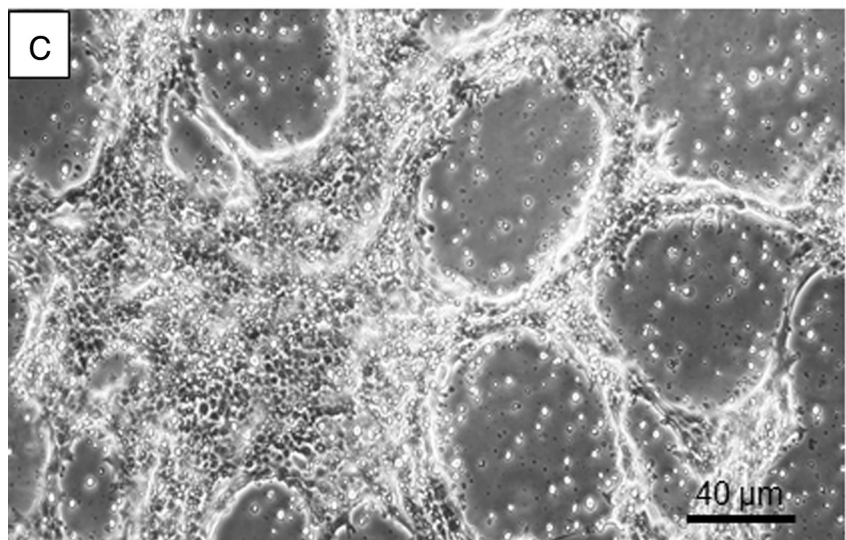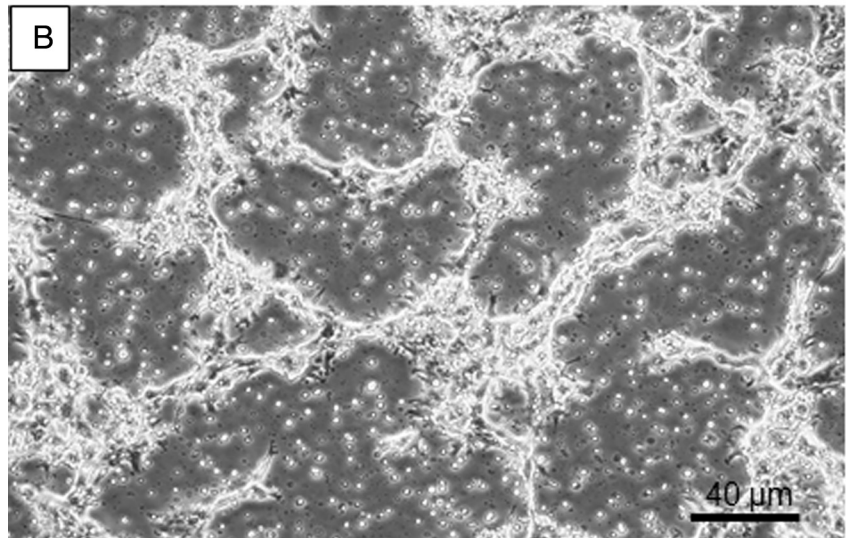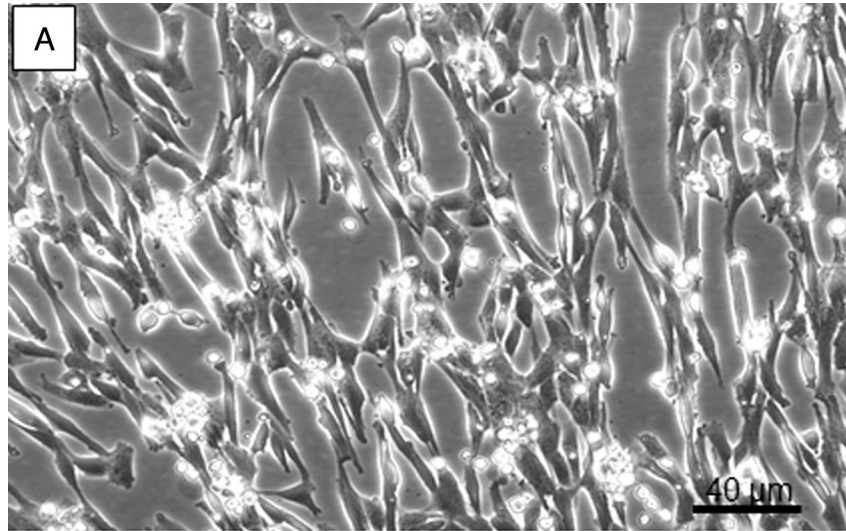
3. Results and discussion

The cytopathic effect was characterized by cell rounding and/or partial or total lysis of the monolayers in comparison to control (Fig. 1A, B and C, respectively). To construct the phylogenetic tree, the sequences of four viruses were included. A fragment of 583 bp corresponding to the VP2CPV gene was amplified in 67 out of 100 (67%) analyzed samples. Nucleotide sequencing confirmed the CPV identity. Forty positive samples (59.7%) belonged to animals that had free access to fragmented forest. Interestingly, 33 out of 67 positive samples (49.2%), were obtained from dogs that have free access to forest, being considered an interface to human-wildlife. The amplified DNA fragments were directly sequenced. Sequence analysis showed that 90.2% of the samples carried the amino acid (aa) Glu at the position 426, characteristic of CPV-2c strains, and 65% of these CPV-2c strains showed the substitution. Despite of the fact that CPV-2c was first described in South America in 2007, the high prevalence of CPV-2c strains in Brazil continues nowadays (Pérez et al., 2007; Streek et al., 2009; Calderón et al., 2015; Fontana et al., 2013; Oliveira et al., 2017). Out of the 67 positive samples, 10% were identified as CPV-2a (KP694304) and 7% CPV-2b (KP694306) (Fig. 2). An additional 50 samples were related to CPV-2c sequences (KP694303 and KP694302). Sequence alignment showed that these sequences presented the amino acid substitution (Asp-426 to Glu-426) that characterizes the 2c type (data not shown). The phylogenetic tree was constructed by the use a representative strains which had been characterized as CPV2-c were subjected to full length VP2 amplification and cloning.

Although CVP-2c has been detected in several Brazilian regions, this is the first description of its circulation in rural/wild areas representing human/wildlife interfaces (Costa et al., 2005; Streek et al., 2009; Castro et al., 2010; Fontana et al., 2013; Oliveira et al., 2017). Despite of the availability and use of several commercial vaccines, viral enteritis — including that associated with CPV infection — remains an important problem for Brazilian dogs (Oliveira et al., 2017). In addition, our study showed that CPV isolation is feasible when faeces are processed shortly after collection. This procedure allows for enriching viral preparations for several purposes, including biological studies of viral replication, drug susceptibility and virus-host interactions (Decaro and Buonavoglia, 2012; Parthiban et al., 2011). In a recent study, rural dogs living in households around six Atlantic forests in southeastern Brazil were serologically tested for CPV antibodies and 97% were positive (Curi et al., 2016). Our study provides a confirmatory piece of information about the circulation of CPV in rural areas and reinforce the risk of

Fig. 1. Cytopathic effects in AF-72-infected cells after three consecutive blind passages, as assessed by phase-contrast microscopy. A) Uninfected AF-72 cells used as controls; B) canine parvovirus VR-2016[TM] (ATCC); C) CPV-2c cytopathic effect characterized by disruption of monolayers; Bar, 50 μm.
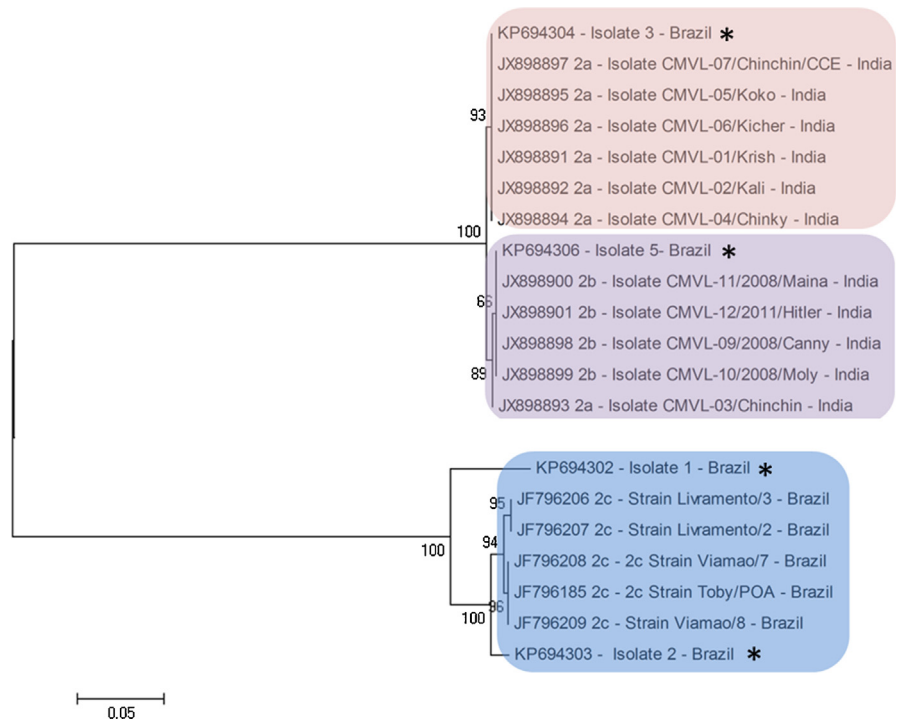
Fig. 2. Phylogenetic analysis of CPV-2a, −b and −c isolated in AF-72 cells based on partial sequencing of the VP2 gene. Evolutionary distances were computed using the Tamura-Nei method and are expressed as the number of base substitutions per site. CPVs detected in rural dogs in this study are denoted with asterisk. Previously published CPV sequences from other sources were included for comparative purposes.

potential transmission of this virus from domestic dogs to wild carnivores. In fact, it is usual see wild carnivores circulating in urban areas due to forest fragmentation in protected areas in Brazil (Curi et al., 2016). In this sense, increased risk of CPV in rural areas in Australia highlighted a need for vigilance in order to prevent virus spread to wild species (Zourkas et al., 2015). Moreover, in Argentina, a re-emergence of CPV-2a in domestic dogs was observed, corroborating the genetic diversity detected worldwide (Calderón et al., 2015). Accurate genotyping for field CPV strains is important for epidemiological issues. Moreover, evaluation of the serological responses suggests vaccination with CPV-2b would cross-protect against CPV-2a and CPV-2c, as well as against CPV-2 which is now extinct in the field (Wilson et al., 2014).

The findings described herein provide relevant information on the potential role of asymptomatic dogs as reservoirs of CPV in rural areas, mainly close related to wildlife carnivores in protected areas of Brazil.

Declarations

Author contribution statement

Flavia V. Viieira, Daniel J. Hoffmann: Conceived and designed the experiments; Performed the experiments.

Carolina U.F. Fabri, Katia D.S. Bresciani, Roberto Gameiro, Eduardo F. Flores: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Tereza C. Cardoso: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

Acosta-Jamett, G., Surot, D., Cortes, M., Marambio, V., Valenzuela, C., Vallverdu, A., Ward, M.P., 2015. Epidemiology of canine distemper and canine parvovirus in domestic dogs in urban and rural areas of the Araucania region in Chile. Vet. Microbiol. 178, 260–264.

Calderón, M.G., Romanutti, C., Wilda, M., D'Antuono, A., Keller, L., Giacomodonato, M.N., Mattion, N., Torre, J.L., 2015. Resurgence of canine parvovirus 2a strain in the domestic dog population from Argentina. J. Virol. Meth. 222, 145–149.

Castro, T.X., Costa, E.M., Leite, J.P.G., Labarthe, N.V., R.C.N.C, Garcia, 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.

Costa, A.P., Leite, J.P.G., Labarthe, N.V., Garcia, R.C.N.C., 2005. Genomic typing of *canine parvovirus* circulating in the State of Rio de Janeiro: Brazil from 1995 to 2001 using polymerase chain reaction assay. Vet. Res. Comm. 29, 735–743.

Curi, N.H., Massara, R.L., Paschoal, A.M.O., Soriano-Araujo, A., Lobato, Z.I., Demetrio, G.R., 2016. Prevalence and risk factors for viral exposure in rural dogs around protected areas of the Atlantic forest. BMC Vet. Res. 12, 21.

Decaro, N., Buonavoglia, C., 2012. Canine parvovirus–a review of epidemiological and diagnostic aspects: with emphasis on type 2c. Vet. Microbiol. 155, 1–12.

Decaro, N., Desario, C., Billi, M., Mari, V., Elia, G., Cavalli, A., Martela, V., Buonavoglia, C., 2011. Western european epidemiological survey for parvovirus and coronavirus infections in dogs. Vet. J. 187, 195–199.

Decaro, N., Desario, C., Parisi, A., Martella, V., Lorusso, A., Miccolupo, A., Mari, V., Colaianni, M.L., Cavalli, A., Di Trani, L., Buonavoglia, C., 2009. Genetic analysis of canine parvovirus type 2c. Virology 385, 5–10.

Fontana, D.S., Rocha, P.R.D., Cruz, R.A.S., Lopes, L.L., Melo, A.L.T., Silveira, M.M., Aguiar, D.M., Pescador, C.A., 2013. A phylogenetic study of canine parvovirus type c in midwestern Brazil. Pesq. Vet. Bras. 32, 214–218.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Series 41, 95–98.

Miranda, C., Thompson, G., 2016. Canine parvovirus: the worldwide occurrence of antigenic variants. J. Gen. Virol. 97, 2043–2057.

Mohan Raj, J., Mukhopadhyay, H.K., Thanislass, J., Antony, P.X., Pillai, R.M., 2010. Isolation, molecular characterization and phylogenetic analysis of canine parvovirus. Infect. Genet. Evol. 10, 1237–1241.

Oliveira, P.S.B., Cargnelutti, J.F., Masuda, E.K., Fighera, R.A., Kommers, G.D., Silva, M.C., Weiblen, R., Flores, E.F., 2017. Epidemiological, clinical and pathological features of canine parvovirus 2c infection in dogs from Southern Brazil. Pesq. Vet. Bras (In press).

Parthiban, S., Mukhopadhyay, H.M., Panneer, D., Antony, P.X., Pillai, R.M., 2011. Isolation and typing of canine parvovirus in CRFK cell line in Puducherry. South India. Indian J. Microbiol. 51, 456–460.

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. 20, 147–152.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Streek, A.F., Souza, C.K., Gonçalves, K.R., Zang, L., Pinto, L.D., Canal, W., 2009. First detection of canine parvovirus type c in Brazil. Braz. J. Microbiol. 40, 465–469.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Vieira, M.J., Silva, E., Oliveira, J., Vieira, A.L., Decaro, N., Desario, C., Muller, A., Carvalheira, J., Buonavoglia, C., Thompson, G., 2008. Canine parvovirus 2c infection in central Portugal. J. Vet. Diag. Invest. 20, 488–491.

Wilson, S., Illambas, J., Siedek, E., Stirling, C., Thomas, A., Plevová, E., Sture, G., Salt, J., 2014. Vaccination of dogs with canine parvovirus type 2b (CPV-2b) induces neutralising antibody responses to CPV-2a and CPV-2c. Vaccine 32, 5420–5424.

Zourkas, E., Ward, M.P., Kelman, M., 2015. Canine parvovirus in Australia: a comparative study of reported rural and urban cases. Vet. Microbiol. 181, 198–203.