Brazilian Journal of Microbiology (2020) 51:1399–1403
https://doi.org/10.1007/s42770-020-00293-2

VETERINARY MICROBIOLOGY - RESEARCH PAPER

Canine parvovirus 2b in fecal samples of asymptomatic free-living South American coatis (*Nasua nasua*, Linnaeus, 1766)

Caroline Giuseppa Spera[1] · Elis Lorenzetti[1,2,3] · Fernanda Louise Pereira Lavorente[1] · Gustavo de Calasans Marques[4] · Jacqueline Muniz Bisca[4] · Carlos Roberto Teixeira[4] · Amauri Alcindo Alfieri[1,2] · Alice Fernandes Alfieri[1,2]

Received: 29 January 2020 / Accepted: 30 April 2020 / Published online: 14 May 2020

Abstract

Canine parvovirus type 2 (CPV-2) is classified into three subtypes (CPV-2a, CPV-2b, and CPV-2c) and is the main cause of enteritis and myocarditis in young domestic and wild animals. This study aimed to evaluate the presence of CPV-2 in the feces of asymptomatic free-living coatis from Garden Forest Reserve, Palmital city, SP, Brazil. Fecal samples from 21 coatis (both sexes, different ages, and different aspects of feces) were collected in August 2014 and March 2015. The nucleic acid extracted was submitted to a polymerase chain reaction (PCR) assay to amplify a fragment of the VP2 gene of CPV-2. Eight (38%) fecal samples were positive in the PCR assay and were confirmed by sequencing. The 7 nucleotide (nt) sequences analyzed showed 100% nt identity with the prototype strain of CPV-2b (CPV-39 strain). The analysis of the deduced amino acid (aa) sequence revealed the presence of the GAT codon (aa D-Asp) at position 426 of the VP2 viral protein (subtype 2b). This study describes for the first time the identification of CPV-2b in asymptomatic free-living coatis (*Nasua nasua*) and suggests that coatis are susceptible to *Carnivore protoparvovirus 1* infection and are important as a reservoir and an asymptomatic carrier to other wild and domestic animal species.

Keywords Coati · Feces · Infectious disease · Molecular detection · CPV-2b

Introduction

Many infectious agents, including viruses that are important to human and animal health, are maintained in nature by wild animals, which may be responsible for the emergence or re-emergence of infectious diseases [1]. The fragmentation of ecosystems due to anthropic impacts increases the contact between wild and domestic animals and, consequently, the cross-species transmission of viral pathogens [2]. The coati (*Nasua nasua*, Linnaeus, 1766) is a mammal of the *Procyonidae* family that belongs to South American wildlife and is one of the most common wild animals of Brazilian fauna [3]. This species is included in the Red List of Threatened Species by the International Union for Conservation of Nature (IUCN) as "Least Concern (LC)" to the risk of extinction and is not included in the Brazilian list of endangered species [4]. In recent years, the populations of coatis have increased considerably due to the tolerance of the species to anthropogenic disturbances. In urban areas, they find food in the garbage, residences, and parks [5].

Several viruses can determine gastroenteric disorders in domestic and wild animals, including canine parvovirus type 2 (CPV-2) [6]. This virus is responsible for one of the most important infectious diseases in young domestic dogs, causing acute hemorrhagic enteritis, associated with severe leukopenia, and myocarditis in puppies [7, 8]. In wild animals, six

Responsible Editor: Fernando R. Spilki.

✉ Amauri Alcindo Alfieri
alfieri@uel.br

[1] Laboratory of Animal Virology, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Celso Garcia Cid Road, PR455 Km 380, P.O. Box 10011, Londrina, Paraná 86057-970, Brazil

[2] Multi-User Animal Health Laboratory, Molecular Biology Unit, Department of Veterinary Preventive Medicine, Universidade Estadual de Londrina, Celso Garcia Cid Road, PR455 Km 380, P.O. Box 10011, Londrina, Paraná 86057-970, Brazil

[3] Post Graduate Program in Animal Health and Production, Universidade Pitágoras Unopar, Arapongas, Paraná, Brazil

[4] Center for Medicine and Research of Wild Animals, Veterinary Hospital, Universidade Estadual Paulista - Julio de Mesquita Filho, P.O. Box 560, Botucatu, São Paulo 18618-000, Brazil

families in Order *Carnivora* are suspected of being susceptible to CPV-2: *Felidae*, *Canidae*, *Procyonidae*, *Mustelidae*, *Ursidae*, *Ailuridae*, and *Viverridae* [7].

CPV-2 belongs to the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Protoparvovirus, Carnivore protoparvovirus 1* species, and is approximately 25 nm in diameter, and its genome consists of single-stranded DNA [9]. The CPV-2 genome is approximately 5.2 kb and encodes four proteins, two structural (VP1 and VP2/VP3), and two nonstructural (NS1 and NS2) [6]. CPV-2 emerged in the mid-1970s as a variant of feline panleukopenia virus (FPV) that adapted to the canine host after acquiring mutations, which allowed it to bind to canine transferrin receptor type 1 (TfR), causing a pandemic infection in dogs around the world. However, CPV-2 was quickly replaced with a new highly virulent variant with some nucleotide (nt) changes in the gene encoding the VP2 coat protein, called CPV-2a [10, 11]. Wild carnivores were important as an intermediate host in the evolution of CPV-2 [12]. Raccoon (*Procyon lotor*), a member of the *Procyonidae* family, is known to be important in the evolution of CPV-2 to CPV-2a [13]. Despite this, CPV-2 had not yet been found in any other species of the same family. Additional evolutions in dogs were responsible for the generation of two new genetic and antigenic variants, CPV-2b and CPV-2c [11].

Therefore, considering the increase in the coati population and the proximity of this species to the urban centers, the aim of this study was to obtain information about the presence of CPV-2 in asymptomatic free-living coatis from Garden Forest Reserve, Palmital city, SP, Brazil.

Materials and methods

Fecal samples

In August 2014 and March 2015, 21 asymptomatic free-living coatis (10 males and 11 females) that live in Garden Forest Reserve (22°48'S, 50°16'W) in the city of Palmital, São Paulo state, Brazil, were captured inside a feeding cage using a hand net. According to the age group, the coatis were classified as pup ($n = 1$), young ($n = 8$), adult ($n = 11$), and elderly ($n = 1$) (Table 1).

To evaluate the physical conditions, the coatis were transferred to press cages, sedated, heavily, and microchip-labeled. Individual fecal samples were collected directly from the rectum with gloves. According to the aspect, the fecal samples were classified into three categories: normal ($n = 13$), pasty with mucus ($n = 6$), and pasty without mucus ($n = 2$) (Table 1). The fecal samples were stored at − 80 °C until processing.

Nucleic acid extraction

Nucleic acid extraction from fecal samples was performed using a combination of the phenol/chloroform/isoamyl alcohol (25:24:1) and silica/guanidinium isothiocyanate nucleic acid extraction methods [14]. In all procedures, aliquots of sterile ultrapure water were included as negative controls, and CPV-2-positive fecal samples were included as positive controls.

Polymerase chain reaction

A PCR assay for CPV-2 was performed with primers that amplify a product with 583 bp of the gene encoding the viral capsid VP2 protein [15]. PCR amplicons were analyzed through electrophoresis on a 2% agarose gel in TBE buffer pH 8.4 (89 mM Tris, 89 mM boric acid, and 2 mM EDTA) containing ethidium bromide (0.5 μg/mL) and visualized under UV light.

Sequencing

PCR products were purified using the commercial PureLink Quick Gel Extraction and PCR Purification Combo kit (Invitrogen Life Technologies, Carlsbad, CA, USA), quantified in Qubit Fluorometer (Invitrogen Life Technologies, Eugene, OR, USA), and sequenced with BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) in an ABI3500 Genetic Analyzer automatic sequencer. The quality of the sequences obtained was evaluated in PHRED, and the contig assembly was obtained with CAP3 software (http://asparagin.cenargen.embrapa.br/phph/). The nt sequences obtained were compared with nt sequences deposited in public databases (GenBank) to verify the similarity. Multiple and pairwise alignments were performed using ClustalW in MEGA software. The nt sequence identity matrix was performed in BioEdit version 7.2.6.1. The nt sequences obtained in the present study were deposited in GenBank under the following accession numbers: MK754060 to MK754066.

Results

A 583-bp fragment of the CPV-2 VP2 gene was amplified in 8 (38%) of the 21 analyzed fecal samples from asymptomatic free-living coatis (Table 1). Out of CPV-2-positive coatis, 4 were young (2 females and 2 males), 3 were adults (2 females and 1 male), and 1 was elderly (male). Four coatis presented normal fecal samples, 1 was pasty without mucus, and 3 were pasty with mucus.

The presence of CPV-2 was confirmed by sequencing the 8 PCR amplicons, of which 7-nt sequences were used for

Table 1 Detection of CPV-2 by PCR assay from fecal samples of asymptomatic free-living coatis (*Nasua nasua*) according to the collection date, gender, estimated age, weight, and aspect of the feces evaluated

Collection date	Gender	Estimated age	Weight (kg)	Feces	PCR results CPV-2
08/02/2014	F	Adult	5.00	Normal	+
08/02/2014	F	Young	2.30	Pasty with mucus	+
08/02/2014	M	Elderly	7.20	Normal	+
08/02/2014	M	Young	2.60	Normal	+
08/02/2014	F	Young	5.10	Pasty with mucus	+
08/02/2014	M	Adult	5.30	Pasty without mucus	–
08/02/2014	M	Adult	5.40	Normal	–
08/02/2014	F	Adult	3.90	Pasty with mucus	+
08/16/2014	F	Adult	3.90	Normal	–
08/16/2014	F	Young	3.00	Normal	–
08/16/2014	M	Young	3.10	Normal	–
08/16/2014	M	Young	3.00	Normal	–
08/16/2014	F	Young	3.90	Pasty with mucus	–
08/16/2014	F	Adult	3.70	Pasty with mucus	–
08/16/2014	M	Young	2.20	Normal	+
08/16/2014	M	Adult	5.10	Pasty without mucus	+
03/20/2015	F	Adult	4.20	Normal	–
03/20/2015	F	Adult	3.90	Normal	–
03/20/2015	M	Adult	6.20	Normal	–
03/20/2015	M	Pup	1.80	Pasty with mucus	–
03/20/2015	F	Adult	4.90	Normal	–

F, female; M, male; (+), positive sample; (−), negative sample

analysis. The CPV-2 strains described in the present study exhibited 100% nt identity with the CPV-2b prototype strain (CPV-39 strain) and 99.6 to 100% nt identity with other CPV-2b strains deposited in GenBank. The deduced amino acid (aa) sequence analysis revealed the presence of the GAT codon (aa D-Asp) at position 426 of the VP2 protein, confirming that the CPV strains detected in coatis belong to subtype 2b (Table 2).

Discussion

Our study provides evidence that coatis (*Nasua nasua*) are susceptible to *Carnivore protoparvovirus 1* infection and can be an important reservoir and an asymptomatic carrier of the virus. From an epidemiological point of view, many infectious agents, including viruses that are important to human and animal health, are carried in nature by wild animals, which may be responsible for the emergence or re-emergence of infectious diseases [1]. The isolation of a population in a controlled space triggers the increase in inbreeding and consequently leads to an extreme monomorphism; however, this may increase the vulnerability of the animals to infectious diseases [16]. Furthermore, the coexistence between domestic and wild animals has increased in recent decades as a consequence of anthropic impacts, such as ecosystem fragmentation and advances in farming, facilitating the cross-species transmission of viral pathogens [2].

Wild carnivores were essential for the emergence and evolution of CPV-2 because several species are reservoirs of *Carnivore protoparvovirus 1* [17–19]. CPV-2 emerged as a variant of the FPV-like lineage, known to infect wild and domestic animals in the order *Carnivora*, such as large and small cats, minks, raccoons, and foxes [20]. FPV acquired the capacity to infect domestic dogs after a mutation in the capsid protein (VP2) occurred, giving it the ability to bind to canine transferrin receptor and resulting in the CPV-like lineage [11]. Raccoons (*Procyon lotor*) are known to be one of the most important wild carnivores in the evolution of CPV-2 [13]. The raccoons are susceptible to FPV and harbor an intermediary virus (*Raccoon Parvovirus*) between CPV-2 and CPV-2a that was not able to infect dogs. However, after genetic and antigenic variations such as a mutation in VP2 protein (87Leu and 101Thr), which are suspected to be crucial, the CPV-2 variant was replaced by a new highly virulent variant, CPV-2a [13]. Raccoons were reported as not susceptible to CPV-like lineage infections, only to FPV-like lineage infections [21]; however, recent studies have reported the isolation of CPV-2 in this species [19, 22]. Thus, although the importance of raccoons as key hosts in the evolution of CPV is known, until now no

Table 2 Alignment of the deduced amino acid sequences of the PCR-amplified VP2 gene fragment (aa 407–440)

Sequence	Amino acid position		
GenBank accession number	407	426	440
CPV-2 (M38245)	GRYPEGDWIQNINFNLPVT**N**DNVLLPTDPIGGKT		
CPV-2a (M24003)	. . . . . **N**		
CPV-2b (M74849)	. . . . . **D**		
CPV-2c (FJ222821)	. . . . . **E**		
BRA-UEL *Nasua nasua* 1 (MK754060)	. . . . . **D**		
BRA-UEL *Nasua nasua* 2 (MK754061)	. . . . . **D**		
BRA-UEL *Nasua nasua* 3 (MK754062)	. . . . . **D**		
BRA-UEL *Nasua nasua* 7 (MK754063)	. . . . . **D**		
BRA-UEL *Nasua nasua* 8 (MK754064)	. . . . . **D**		
BRA-UEL *Nasua nasua* 9 (MK754065)	. . . . . **D**		
BRA-UEL *Nasua nasua* 10 (MK754066)	. . . . . **D**		

The aligned sequences correspond to a CPV-2 vaccine strain (CPV-b, M38245), a CPV-2a strain (CPV-15, M24003), a CPV-2b strain (CPV-39, M74849), and a CPV-2c strain (56/00, FJ222821). Sequences from this study were deposited in GenBank with MK754060 to MK754066 accession numbers. Position 426 is highlighted in bold

other member of the *Procyonidae* family is known to be susceptible to FPV-like lineage or CPV-like lineage infections.

The analysis of the fecal samples of the coatis (*Nasua nasua*) from this study revealed the presence of CPV-2b in 8 out of 21 (38%) animals evaluated that inhabit a controlled space in Garden Forest Reserve in Palmital, SP, Brazil. To the best of our knowledge, this is the first study to report the presence of CPV-2b in asymptomatic free-living coatis. Until now, only a member of the *Procyonidae* family, the raccoon (*Procyon lotor*), was reported to be susceptible to CPV-2 infection [19, 22]. However, the CPV found in coatis (CPV-2b) is different from that in raccoons. CPV-2b is considered a common subtype detected in the world population of domestic dogs in recent years, including in Brazil [7]. Studies have shown a positive relationship between the subtype circulating among dogs and wild animals, reinforcing the importance of domestic dogs as reservoirs of the virus for wild animal infection [18, 19].

Thus, the close contact of the coatis (*Nasua nasua*) with dogs or feces of symptomatic or asymptomatic animals living near Garden Forest Reserve may have been responsible for the contamination of the coatis. The CPV-2 viral particle is small and has no glycoprotein envelope and is considered one of the most resistant viruses in the environment. These characteristics are associated with the presence of a high viral titer (10^9 particles per gram) in the feces of animals with acute infection and contribute to the horizontal transmission of the virus, where the direct contact of a symptomatic or an asymptomatic animal with a susceptible animal is not necessary [23].

The coatis were apparently healthy at the time of the sampled, but they were not clinically evaluated at other times; therefore, it was not possible to determine whether clinical signs of CPV-2b infection were demonstrated. Although raccoons are clinically susceptible to CPV-2, which leads to loss of appetite and severe diarrhea [22], it is not possible to infer that CPV-2b can cause disease in coatis or if coatis are only asymptomatic carriers. The coatis were part of a mixed population of 21 animals of both sexes, different age groups (pup, young, adult, and elderly), and different aspects of feces (normal, pasty without mucus, and pasty with mucus), indicating that these factors were not decisive to the asymptomatic carrier state.

Only the fecal samples collected in August 2014 were positive for CPV-2b. In that month, the average temperature was 24.4 °C, preceded by the months of June and July, which registered the lowest temperatures of 2014, 23.5 °C and 22.2 °C, respectively (Annual Climatological Bulletin of the Station IAG/USP 2014), in accordance with studies that have shown that the shedding of viral particles varies seasonally and increases in the winter and decreases in the summer. This may occur due to degradation at high temperatures, causing CPV-2b to reach nondetectable levels [24].

Conclusion

Therefore, this is the first report of CPV-2b in a population of asymptomatic free-living coatis (*Nasua nasua*), reinforcing the importance of asymptomatic small carnivores in the maintenance of viruses in the environment and the role of the transmission of viruses in both domestic and wild populations.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was carried out following with the Ethical Principles adopted by the Brazilian College of Animal Experimentation (COBEA). The methodology adopted in the study was approved by the Animal Experimentation Ethics Chamber of the Faculty of Veterinary Medicine and Animal Science of the Universidade Estadual Paulista (UNESP), Botucatu Campus (N°09/2015-CEUA) and SISBIO N°47767-1. The coatis (*Nasua nasua*) evaluated in this study are part of the project of Population Control and Zoo Sanitary Survey developed by the Faculty of Veterinary Medicine and Animal Science (FMVZ) of UNESP, organized and coordinated by the Center for Medicine and Research of Wild Animals (CEMPAS), and authorized by the State Department of the Environment in partnership with Palmital City (N°119/2014, in situ management authorization; N°11519/2013, SMA/DeFau Process; N°12982/2012, related process).

References

1. Allison AB, Kohler DJ, Ortega A, Hoover EA, Grove DM, Holmes EC, Parrish CR (2014) Host-specific parvovirus evolution in nature is recapitulated by in vitro adaptation to different carnivore species. PLoS Pathog 10(11):e1004475. https://doi.org/10.1371/journal.ppat.1004475
2. Deem SL, Karesh WB, Weisman W (2001) Putting theory into practice: wildlife health in conservation. Conserv Biol 15(5):1224–1233. https://doi.org/10.1111/j.1523-1739.2001.00336.x
3. Gompper ME, Decker DM (1998) *Nasua nasua*. Mamm Species 580:1–9. https://doi.org/10.2307/3504444
4. Emmons L, Helgen K (2016) *Nasua nasua*. The IUCN Red List of Threatened Species 2016. https://doi.org/10.2305/IUCN.UK.2016-1.RLTS.T41684A45216227.en
5. Alves-Costa CP, Eterovick PC (2007) Seed dispersal services by coatis (*Nasua nasua, Procyonidae*) and their redundancy with other frugivores in southeastern Brazil. Acta Oecol 32(1):77–92. https://doi.org/10.1016/j.actao.2007.03.001
6. Nandi S, Kumar M (2010) Canine parvovirus: current perspective. Indian J Virol 21(1):31–44. https://doi.org/10.1007/s13337-010-0007-y
7. Miranda C, Thompson G (2016) Canine parvovirus: the worldwide occurrence of antigenic variants. J Gen Virol 97(9):2043–2057. https://doi.org/10.1099/jgv.0.000540
8. Ford J, McEndaffer L, Renshaw R, Molesan A, Kelly K (2017) Parvovirus infection is associated with myocarditis and myocardial fibrosis in young dogs. Vet Pathol 54(6):964–971. https://doi.org/10.1177/0300985817725387
9. ICTV (2018) International Committee on Taxonomy of Viruses. ICTV 2018 Master Species List (MSL33). https://talk.ictvonline.org/files/master-species-lists/m/msl/7992. Accessed 7 January 2019
10. Buonavoglia C, Martella V, Pratelli 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(Pt 12):3021–3025. https://doi.org/10.1099/0022-1317-82-12-3021
11. Hoelzer K, Parrish CR (2010) The emergence of parvoviruses of carnivores. Vet Res 41(6):39. https://doi.org/10.1051/vetres/2010011
12. Decaro N, Buonavoglia C (2012) Canine parvovirus - a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Vet Microbiol 155(1):1–12. https://doi.org/10.1016/j.vetmic.2011.09.007
13. Allison AB, Harbison CE, Pagan I, Stucker KM, Kaelber JT, Brown JD, Ruder MG, Keel MK, Dubovi EJ, Holmes EC, Parrish CR (2012) Role of multiple hosts in the cross-species transmission and emergence of a pandemic parvovirus. J Virol 86(2):865–872. https://doi.org/10.1128/JVI.06187-11
14. Alfieri AA, Parazzi ME, Takiuchi E, Médici KC, Alfieri AF (2006) Frequency of group a rotavirus in diarrhoeic calves in Brazilian cattle herds, 1998-2002. Trop Anim Health Prod 38(7–8):521–526. https://doi.org/10.1007/s11250-006-4349-9
15. 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 Investig 19(5):535–539. https://doi.org/10.1177/104063870701900512
16. O'Brien SJ, Roelke ME, Marker L, Newman A, Winkler CA, Meltzer D, Colly L, Evermann JF, Bush M, Wildt DE (1985) Genetic basis for species vulnerability in the cheetah. Science 227(4693):1428–1434. https://doi.org/10.1126/science.2983425
17. Steinel A, Parrish CR, Bloom ME, Truyen U (2001) Parvovirus infections in wild carnivores. J Wildl Dis 37(3):594–607. https://doi.org/10.7589/0090-3558-37.3.594
18. Battilani M, Scagliarini A, Tisato E, Turilli C, Jacoboni I, Casadio R, Prosperi S (2001) Analysis of canine parvovirus sequences from wolves and dogs isolated in Italy. J Gen Virol 82(Pt 7):1555–1560. https://doi.org/10.1099/0022-1317-82-7-1555
19. Allison AB, Kohler DJ, Fox KA, Brown JD, Gerhold RW, Shearn-Bochsler VI, Dubovi EJ, Parrish CR, Holmes EC (2013) Frequent cross-species transmission of parvoviruses among diverse carnivore hosts. J Virol 87(4):2342–2347. https://doi.org/10.1128/jvi.02428-12
20. Steinel A, Munson L, van Vuuren M, Truyen U (2000) Genetic characterization of feline parvovirus sequences from various carnivores. J Gen Virol 81(Pt 2):345–350. https://doi.org/10.1099/0022-1317-81-2-345
21. Appel MJ, Parrish CR (1982) Raccoons are not susceptible to canine parvovirus. J Am Vet Med Assoc 181(5):489
22. Kapil S, Rezabek G, Germany B, Johnston L (2010) Isolation of a virus related to canine parvovirus type 2 from a raccoon (*Procyon lotor*). Vet Rec 166(1):24–25. https://doi.org/10.1136/vr.b5587
23. Berns K, Parrish CR (2007) Parvoviridae. In: Knipe DM, Howley PM (eds) Fields Virology, 5th edn. Lippincott Williams and Wilkins, Philadelphia, pp 2437–2477
24. Mech LD, Almberg ES, Smith D, Goyal S, Singer RS (2012) Use of real-time PCR to detect canine parvovirus in feces of free-ranging wolves. J Wildl Dis 48(2):473–476. https://doi.org/10.7589/0090-3558-48.2.473