

## **ABSENCE OF PARVOVIRUS SHEDDING IN FECES OF THREATENED CARNIVORES FROM MISIONES, ARGENTINA**

Authors: Maria Marcela Orozco, Danilo Bucafusco, Hernán Dario Argibay, Miguel Angel Rinas, Karen Elizabeth DeMatteo, et. al.

Source: Journal of Zoo and Wildlife Medicine, 49(4) : 1054-1060

Published By: American Association of Zoo Veterinarians

URL: <https://doi.org/10.1638/2016-0301.1>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non-commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

## ABSENCE OF PARVOVIRUS SHEDDING IN FECES OF THREATENED CARNIVORES FROM MISIONES, ARGENTINA

Maria Marcela Orozco, D.V.M., Ph.D., Danilo Bucafusco, D.V.M., Ph.D., Hernán Dario Argibay, D.V.M., Ph.D., Miguel Angel Rinas, D.V.M., Karen Elizabeth DeMatteo, B.Sc., Ph.D., Carina Francisca Argüelles, Ph.D., Ana Cristina Bratanich, D.V.M., Ph.D., and Ricardo Esteban Gürtler, B.Sc., Ph.D.

**Abstract:** Since its emergence in the 1970s, canine parvovirus (CPV) has spread worldwide and infects a wide variety of mammalian hosts, including domestic and nondomestic carnivores. Today it is one of the most important pathogenic viruses associated with high morbidity and mortality in domestic dogs (*Canis familiaris*). In South America, the range of wild hosts has been scarcely studied and the epidemiology of CPV in wildlife is still unclear. In 2011, feces from five wild carnivores (bush dog [*Speothos venaticus*], jaguar [*Panthera onca*], puma [*Puma concolor*], oncilla [*Leopardus guttulus*], and ocelot [*Leopardus pardalis*]) were collected in Misiones, Argentina, using a detection dog. Of the 289 feces collected, 209 (72.3%) had sufficient sample remaining to be used in this study and the majority of these were genetically confirmed to individual (81.3%) and sex (78.4%) level. In fact, these samples represent a minimum of 115 individuals (10 jaguars, 13 pumas, 33 ocelots, 38 oncillas, and 21 bush dogs). Through polymerase chain reaction, a 583-bp fragment in the VP2 gene of CPV was amplified in these samples. While no samples showed evidence of infection, this does not exclude the occurrence of CPV in wild carnivores in the area, as intermittent viral shedding could condition the diagnosis of CPV in feces of infected wild mammals. Locally, it is recommended that long-term monitoring of parvovirus be continued in wildlife and expanded to domestic carnivores. Internationally, this study provides a useful contribution to the approach to the sylvatic cycle of parvovirus in wild carnivores.

**Key words:** *Leopardus guttulus*, *Leopardus pardalis*, *Panthera onca*, parvovirus, *Puma concolor*, *Speothos venaticus*.

### BRIEF COMMUNICATION

Since domestic animals can act as reservoirs for a variety of pathogens that can be transmitted to wild carnivores, they put the conservation of species worldwide at risk.<sup>11</sup> The exponential increase in human populations and their expan-

sion into wild habitats is associated with a concurrent expansion of domestic animals and their pathogens.<sup>2</sup> Several infectious diseases that affect domestic dogs (*Canis familiaris*) can cause mortality in wildlife, including canine distemper virus, canine adenovirus, rabies, and canine parvovirus (family Parvoviridae, genus *Protoparvovirus*).<sup>3</sup> Canine parvovirus (CPV) is endemic in domestic dog populations worldwide. Since its initial description in 1978,<sup>6</sup> CPV has spread globally as a new pandemic disease of domestic dogs and is characterized by acute hemorrhagic enteritis and myocarditis with high morbidity and mortality rates.<sup>30</sup>

From the Universidad de Buenos Aires, Consejo Nacional de Investigaciones Científicas y Técnicas, Instituto de Ecología, Genética y Evolución de Buenos Aires, Facultad de Ciencias Exactas y Naturales, Intendente Güiraldes 2160, Ciudad Universitaria, Pabellón 2, 2° piso, (C1428EHA), Buenos Aires, Argentina (Orozco, Argibay, Gürtler); the Universidad de Buenos Aires, Facultad de Ciencias Veterinarias, Área de Virología, Av. Chorroarín 170, (C1427CWN), Buenos Aires, Argentina (Bucafusco, Bratanich); the Parque Ecológico El Puma, Ministerio de Ecología y Recursos Naturales Renovables, San Lorenzo 1538, (3300), Posadas, Misiones, Argentina (Rinas); the WildCare Institute at the Saint Louis Zoo, 1 Government Drive, St. Louis, MO 63110, USA, and Department of Biology, Washington University in St. Louis, 1 Brookings Drive, Box 1137, St. Louis, MO 63130, USA (DeMatteo); and the Grupo de Investigaciones en Genética Aplicada, Instituto de Biología Subtropical, Nodo Posadas, Universidad Nacional de Misiones (UNaM)–Consejo Nacional de Investigaciones Científicas y Técnicas, Jujuy 1745 (3300), Posadas, Misiones, Argentina (Argüelles). Correspondence should be directed to Dr. Orozco (marcelaorozco.vet@gmail.com).

Some carnivore parvoviruses, such as the feline panleukopenia virus (FPV), minute virus of canines, mink enteritis virus, and raccoon parvovirus, have been known for many years.<sup>31</sup> Several studies suggest that CPV emerged from a FPV-like virus of domestic cats or from a closely related virus of wildlife species.<sup>4</sup> In the last 40 yr, novel antigenic and genetic variants of CPV have emerged as a result of evolutionary selections in nature.<sup>27,30</sup> Coyotes (*Canis latrans*) and wolves (*Canis lupus*) are susceptible to natural infection with CPV.<sup>29</sup> In fact, in *C. lupus* CPV has been associated with a reduction in early pup survival and limited population growth.<sup>21</sup>

The new antigenic variants can change their host range, infecting other species including felines.<sup>23</sup> Analyses of parvovirus DNA sequences recovered from wild carnivores have demonstrated that 95% were derived from CPV-like viruses.<sup>4,5</sup> In fact, a recent US study that sampled 852 free-ranging wild carnivores showed that through only a few mutations in the capsid protein, parvoviruses may cross species barriers to infect less susceptible hosts.<sup>5</sup> In this study, genetic sequences of CPV were identified in 8 of the 18 studied species, with the highest prevalence found in pumas (67%), gray wolves (43%), coyotes (24%), fishers (*Martes pennanti*; 20%), raccoons (*Procyon lotor*; 19%), and bobcats (*Lynx rufus*; 16%). These high rates of prevalence suggest the possibility of sustained transmission in natural environments.<sup>5</sup>

While CPV appears to be widespread among North American carnivores,<sup>5</sup> in other areas little is known about its host range and the sylvatic cycles of the virus. The Atlantic Forest and Gran Chaco in South America are highly diverse and contain a wide variety of large species, including endangered and threatened carnivores. In both areas, deforestation rates are exceptionally high and forest fragments are under intense pressure.<sup>34</sup> In South America most reports on CPV prevalence in wild carnivores has been based on serologic surveys.<sup>1,18,20,25,26</sup> However, serum samples can be difficult to obtain since they require physical capture of the animal and results only reflect pathogen exposure. In contrast, the use of molecular detection of CPV in feces of wild carnivores reflects the shedding of viral particles and indicates the prevalence of infection in the area.

This study aimed to assess CPV occurrence in wild carnivore feces found in and around protected areas in Misiones, Argentina, with a goal to identify areas with high levels of CPV transmission and contribute to management strategies. To examine CPV in Misiones, this study used noninvasive samples (i.e., feces) collected using detection dogs, a technique that allows the number of samples and total area covered to be maximized.<sup>12,13</sup>

Detection dog surveys were conducted from May to August 2011 in and around 12 protected areas in the northern-central zones of Misiones, Argentina, with 30% of the area surveyed partially or completely outside of protected areas and the remainder at varying distances from edge habitat depending on the size of the protected area (Fig. 1).<sup>12,13</sup> The protected areas are mainly composed of Upper Paraná Atlantic Forest but vary in their

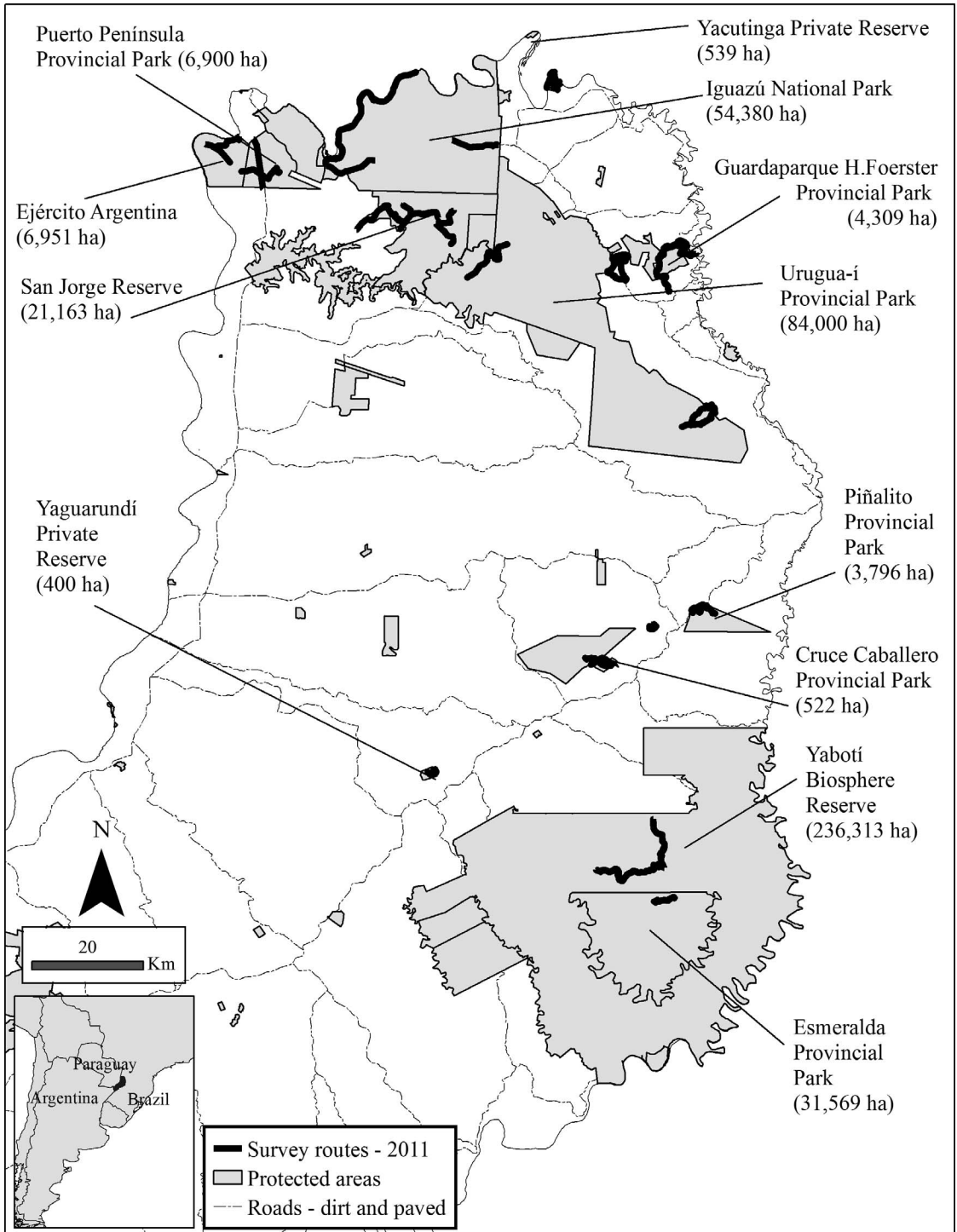
size (Fig. 1) and degree of protection. They are surrounded by a mixture of native forest, monoculture plantations, subsistence farming, small villages, livestock, and free-ranging domestic animals. Five carnivores were targeted in the surveys: bush dog (*Speothos venaticus*), jaguar (*Panthera onca*), puma (*Puma concolor*), oncilla (*Leopardus guttulus*), and ocelot (*Leopardus pardalis*).<sup>12,13</sup>

Of the 289 feces collected, 209 (72.3%) had sufficient sample remaining to be used in this study and the majority of these were genetically confirmed to individual (81.3%) and sex (78.4%) level (Table 1).<sup>12,13</sup>

While exact sample locations are not reported or displayed per government request as a precaution to protect these threatened and endangered carnivores from targeted poaching, a summary of general location and the genetic results are presented in Table 1. In total 115 unique individuals were identified: 10 jaguars, 13 pumas, 33 ocelots, 38 oncilla, and 21 bush dogs.<sup>12,13</sup>

Once collected, samples were stored at a minimum of  $-20^{\circ}\text{C}$ . In the lab, DNA was extracted using phenol-chloroform combined with a standard ethanol precipitation. Polymerase chain reaction (PCR) amplifications of a 583-bp fragment of the VP2 gene were carried out to diagnose CPV presence.<sup>9</sup> Each DNA sample was analyzed three times (pure or undiluted, diluted 1 : 50, and diluted 1 : 100) totaling 627 PCR amplifications. A primer pair (555 forward : 555 reverse), which anneals to the majority of reported CPV and FPV-like sequences, was used in the PCR amplifications. Each 50- $\mu\text{l}$  reaction was composed of 1 $\times$  buffer (KCL 50 mM, Tris-HCl 10 mM, pH 8.3), 1.5 mM MgCl<sub>2</sub>, 10  $\mu\text{M}$  of each deoxynucleotide (dATP, dCTP, dGTP, dTTP), 25  $\mu\text{M}$  of each primer, 1.25 U of GoTaq, and 2  $\mu\text{l}$  of DNA template. The cycling conditions consisted of an initial denaturation at  $94^{\circ}\text{C}$  for 5 min; 40 cycles of denaturation at  $94^{\circ}\text{C}$  for 30 sec, annealing at  $50^{\circ}\text{C}$  for 1 min, and polymerization at  $72^{\circ}\text{C}$  for 1 min; and a final extension at  $72^{\circ}\text{C}$  for 10 min. PCR products were visualized by electrophoresis through an 1% agarose gel stained with ethidium bromide. Reference CPV-d (CPV-2 strain)<sup>28</sup> and a local CPV-2C isolate were used as positive controls in all assays.

All 209 samples, from a minimum of 115 individuals, were negative for CPV. These results represent the first assessment of the occurrence of parvovirus in feces of endangered carnivores in Argentina and indicate an absence of CPV in the evaluated environments at the time of the study.



**Figure 1.** Location of Misiones, Argentina, in South America (inset). Map of Misiones with 2011 survey routes shown relative to protected areas and roads.

**Table 1.** Summary of 209 feces (species) and 170 genotyped scats (individual) found in protected and unprotected areas in Misiones, Argentina. The number of scats identified to species level and individual level, as well as the number of unique individuals (no. of individuals) is reported. Sexes for the 115 unique individuals are shown.

Location	Bush dog						Jaguar						Puma						Ocelot						Oncilla						
	No. of scats			No. of individuals			No. of scats			No. of individuals			No. of scats			No. of individuals			No. of scats			No. of individuals			No. of scats			No. of individuals			
	S	I	U	M	F	U	S	I	U	M	F	U	S	I	U	M	F	U	S	I	U	M	F	U	S	I	U	M	F	U	
Iguazú NP <sup>a</sup>							11	10	1	3	0	0	9	9	5	0	0	0	16	13	6	4	0	0	1	1	1	1	1	0	0
Uruguá-i PP							1	0	—	—	—	—	4	2	2	0	0	0	5	5	1	2	0	0	5	4	3	0	0	0	0
Reserva San Jorge	1	0	—	—	—	—	—	—	—	—	—	—	3	2	1	0	0	0	5	3	2	0	1	15	12	4	6	2	2	0	0
Puerto Península PP	5	4	1	2	0	—	—	—	—	—	—	—	3	3	2	0	0	0	2	1	1	1	0	0	13	11	6	1	0	0	0
Esmeralda PP & Yabotí Biosphere Reserve	3	3	3	0	0	0	2	2	1	0	0	0	2	0	—	—	—	—	14	10	3	0	2	4	3	1	2	0	0	0	0
Military Area – Ejército Uruguá-i PP & San Jorge Reserve	1	1	1	0	0	0	2	2	0	2	0	0	2	2	0	1	0	0	—	—	—	—	—	—	5	4	3	1	0	0	0
Iguazú NP, San Jorge Reserve, & outside protected areas							5	5	1	0	0	0	5	5	1	0	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—
Cruce Caballero PP																															
Guardaparque H. Foerster PP	1	0	—	—	—	—	—	—	—	—	—	—	3	3	2	0	0	0	8	6	2	1	0	4	2	1	1	0	0	0	0
Yacutinga Private Reserve																			13	13	2	3	1	—	—	—	—	—	—	—	—
Piñalito PP	3	3	2	1	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Outside protected areas	16	12	6	4	0	0	1	1	0	1	0	0	—	—	—	—	—	—	2	2	0	2	0	9	8	3	3	0	0	0	0
Total	31	24	13	8	0	0	29	25	4	6	0	0	26	21	12	1	0	0	65	53	17	12	4	58	47	22	14	2	2	2	2

<sup>a</sup> NP indicates National Park; PP, Provincial Park; S, scats identified to species level; I, scats identified to individual level, M, male; F, female; U, unknown sex. — indicates that gender determination was not possible for this sample. A blank cell indicates that a sample was not collected.



Using conventional and real-time PCRs, studies in North America and Europe have detected the genome of closely related CPV in tissues and feces of several wild carnivores; however, prevalence varied widely.<sup>4,15,16</sup> In Europe the exposure of wild carnivores to parvovirus was demonstrated with a fairly widespread geographic distribution.<sup>15,16</sup> In Bulgaria, only 4% of wolves and red foxes (*Vulpes vulpes*)<sup>16</sup> were determined to be positive and infected by CPV-2a strains. This is in sharp contrast to the 20% observed in a variety of US mammals, with pumas reaching a prevalence of 67%,<sup>5</sup> and the notable incidence of parvovirus found in red foxes (79%) and Egyptian mongooses (*Herpestes ichneumon*; 58%) in Portugal.<sup>15</sup> In all these studies the virus could not be isolated, suggesting that the findings could correspond to previous infections.

An Italian study identified antigenically and genetically identical strains in domestic dogs and wolves.<sup>7</sup> In the United States, phylogenetic analyses provided evidence of sustained onward transmission and frequent cross-species transmission even bidirectionally between domestic and wild species.<sup>4,5</sup> Although it is not clear which wild species act as natural reservoirs for CPV-related viruses, these studies demonstrate the circulation of CPV in wild habitats. There remains the need to investigate host susceptibility and transmissibility of CPV to new hosts.

CPV has never been studied systematically in South America using molecular tools; however, there have been regional studies focused on the variants affecting domestic dogs and the role of rural dogs as reservoirs for infectious diseases for wild carnivores. A recent study in the center and north of Argentina showed the occurrence of CPV2a, CPV2b, and CPV2c in domestic dogs.<sup>19</sup> Preliminary results of CPV seroprevalence in rural dogs in South America indicates that there is broad variation with only 2% positive in Santiago del Estero, Argentina<sup>25</sup> compared to >95% positive in Isoso, Bolivia.<sup>17</sup> In Brazil, prevalence and risk factors for CPV exposure in rural dogs were described<sup>10</sup> and CPV was isolated in cell culture from 67% of the sampled healthy dogs living around protected areas of Atlantic Forest.<sup>33</sup> In Argentina, recent carnivore surveys highlight the fact that dogs and wildlife have potential opportunities for contact.<sup>12-14</sup> This is supported by a serologic survey of CPV using a hemagglutination inhibition assay in protected and disturbed areas in the Chaco province that showed a very high seroprevalence (93.9–94.6%) of CPV antibodies in domestic rural dogs and

similarly high exposure titers to CPV in nearly all wild carnivores and marsupials.<sup>26</sup>

CPV has been confirmed in domestic dogs from Misiones, Argentina, and neighboring areas.<sup>19</sup> While there are no official data on CPV prevalence in domestic dogs from the study area, it is known that the dog population is very abundant. A 2008 survey in Posadas, Misiones, of 1,000 homes, which represents only 1.25% of the total homes ( $n = 80,000$ ), estimated the abundance of domestic dogs at 100,000.<sup>22</sup>

The negative findings across multiple threatened carnivores in an area of increasing fragmentation does not exclude the occurrence of CPV in wild carnivores of Misiones; instead it is assumed that the number of individuals in the active phase of viral replication or at a point where PCR detection of the viral genome in feces is possible, due to the intermittent shedding, would be very low.<sup>8,24,32</sup> While some samples correspond to the same individual, all samples are considered in the analysis because the narrow window of time for CPV release into the feces allows us to analyze samples of the same animal at different times independently without losing validity.

This study analyzed a large number of samples from elusive or rare species collected with a novel methodology. It cannot be affirmed that the data are representative of the entire region given the zero prevalence of CPV that was detected. However, the results could contribute to an improved interpretation of new results or hypotheses on the subject in different scenarios. It is suggested that techniques such as those used in this study should be preferentially applied in future surveys because they provide a realistic picture of actual presence and transmission of CPV in wild populations. In addition, future surveillance studies in Misiones and similar surveys worldwide should not only continue long-term assessments on wild carnivores but also expand to include monitoring of domestic dogs from surrounding areas.

*Acknowledgments:* Funding for this study was provided by Agencia Nacional de Promoción Científica y Tecnológica (PICT 2011-2072 and PICTO-Glaxo 2011-0062), and the University of Buenos Aires (20020100100-944) to REG. MMO, DB, and REG are members of Consejo Nacional de Investigaciones Científicas y Técnicas Researcher's Career. Funding for the collection and genetic analyses of scats was provided by the Conservation, Food, & Health Foundation; a National Geographic Society Conservation Trust

Grant; Palm Beach Zoo Conservation Fund; Paris Zoo; Jaguar Conservation Fund–Woodland Park Zoo; and North of England Zoological Society Conservation and Research Fund. The Ministerio de Ecología y Recursos Naturales Renovables of Misiones (MEyRNR) and the Administración de Parques Nacionales of Argentina provided permits and help with field logistics. The MEyRNR provided housing during the field surveys. Sincere thanks to the zoos that provided training samples for the detection dog and the numerous Argentinian students who assisted in the field, as well as various collaborators and private land owners that helped both in and out of the field. Sincere thanks to A. Allison and C. Parrish for valuable discussions of these results.

### LITERATURE CITED

1. Acosta-Jamett G, Surot D, Cortés M, Marambio V, Valenzuela C, Vallverdu A, Ward MP. Epidemiology of canine distemper and canine parvovirus in domestic dogs in urban and rural areas of the Araucanía region in Chile. *Vet Microbiol.* 2015; 178(3–4):260–264.
2. Aguirre AA. Wild canids as sentinels of ecological health: a conservation medicine perspective. *Parasit Vectors.* 2009;2:Suppl 1:S7.
3. Alexander KA, McNutt JW, Briggs MB, Standers PE, Funston P, Hemson G, Keet, van Vuuren M. Multi-host pathogens and carnivore management in southern Africa. *Comp Immunol Microbiol Infect Dis.* 2010; 33(3):249–265.
4. Allison AB, Kohler DJ, Fox KA, Brown JD, Gerhold RW, Shearn-Bochsler VI, Dubovi EJ, Parrish CR, Holmes EC. Frequent cross-species transmission of parvoviruses among diverse carnivore hosts. *J Virol.* 2013;87(4):2342–2347.
5. Allison AB, Kohler DJ, Ortega A, Hoover EA, Grove DM, Holmes EC, Parrish CR. Host-specific parvovirus evolution in nature is recapitulated by in vitro adaptation to different carnivore species. *PLoS Pathog.* 2014;10(11):e1004475.
6. Appel MJG, Scott FW, Carmichael LE. Isolation and immunization studies of a canine parvo-like virus from dogs with hemorrhagic enteritis. *Vet Res.* 1979; 105(8):156–159.
7. Battilani M, Scagliarini A, Tisato E, Turilli C, Jacoboni I, Casadio R, Prosperi S. Analysis of canine parvovirus sequences from wolves and dogs isolated in Italy. *J Gen Virol.* 2001;82(7):1555–1560.
8. Bouillant A, Hanson RP. Epizootiology of mink enteritis: III. Carrier state in mink. *Can J Comp Med Vet Sci.* 1965;29(7):183–189.
9. Buonavoglia C, Martella V, Pratelli A, Tempesta M, Cavalli A, Buonavoglia D, Bozzo G, Elia G, Decaro N, Carmichael L. Evidence for evolution of canine parvovirus type 2 in Italy. *J Gen Virol.* 2001;82(12): 3021–3025.
10. Curi NHA, Massara RL, Paschoal AMO, Soriano-Araújo A, Lobato ZIP, Demétrio GR, Chiarello AG, Passamani M. Prevalence and risk factors for viral exposure in rural dogs around protected areas of the Atlantic Forest. *BMC Vet Res.* 2016 Jan 28;12:21. doi: 10.1186/s12917-016-0646-3.
11. Daszak P, Cunningham AA, Hyatt AD. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Trop.* 2001;78(2): 103–116.
12. DeMatteo KE, Rinas MA, Argüelles CF, Holman BE, Di Bitetti MS, Davenport B, Parker PG, Eggert LS. Using detection dogs and genetic analyses of scat to expand knowledge and assist felid conservation in Misiones, Argentina. *Integr Zool.* 2014;9(5): 623–639.
13. DeMatteo KE, Rinas MA, Argüelles CF, Zurano JP, Selleski N, Di Bitetti MS, Eggert LS. Noninvasive techniques provide novel insight for the elusive bush dog (*Speothos venaticus*). *Wildl Soc Bull.* 2014;38(4): 862–873.
14. DeMatteo KE, Rinas MA, Zurano JP, Selleski N, Schneider RG, Argüelles CF. Using niche-modelling and species-specific cost analyses to determine a multispecies corridor in a fragmented landscape. *PLoS ONE.* 2017;12(8):e0183648.
15. Duarte MD, Henriques AM, Barros SC, Fagulha T, Mendonça P, Carvalho P, Monteiro M, Fevereiro M, Basto MP, Rosalino LM, Barros T, Bandeira V, Fonseca C, Cunha MV. Snapshot of viral infections in wild carnivores reveals ubiquity of parvovirus and susceptibility of Egyptian mongoose to feline panleukopenia virus. *PLoS ONE.* 2013; 8(3):e59399.
16. Filipov C, Desario C, Patouchas O, Eftimov P, Gruichev G, Manov V, Filipov G, Buonavoglia C, Decaro N. A ten-year molecular survey on parvoviruses infecting carnivores in Bulgaria. *Transbound Emerg Dis.* 2016;63(4):460–464.
17. Fiorello CV, Noss AJ, Deem SL. Demography, hunting ecology, and pathogen exposure of domestic dogs in the Isoso of Bolivia. *Conserv Biol.* 2006;20(3): 762–771.
18. Fiorello CV, Noss AJ, Deem SL, Maffei L, Dubovi EJ. Serosurvey of small carnivores in the Bolivian Chaco. *J Wildl Dis.* 2007;43(3):551–557
19. Gallo Calderón M, Romanutti C, D'Antuono A, Keller L, Mattion N, La Torre J. Evolution of canine parvovirus in Argentina between years 2003 and 2010: CPV2a become the predominant variant affecting the domestic dog population. *Virus Res.* 2011;157(1):106–110.
20. Martino PE, Montenegro JL, Preziosi JA, Venturini C, Bacigalupe D, Stanchi NO, Bautista EL. Serological survey of selected pathogens of free-ranging foxes in southern Argentina, 1998–2001. *Rev Sci Tech OIE.* 2004;23(3):801–806.

21. Mech LD, Goyal SM, Paul WJ, Newton WE. Demographic effects of canine parvovirus on a free-ranging wolf population over 30 years. *J Wildl Dis.* 2008;44(4):824–836.
22. Misiones Institute of Statistics and Censuses (IPEC, Argentina) [Internet]. 2008 [cited 2018 April 23]. Available from <https://ipecmisiones.org/>
23. Mochizuki M, Horiuchi M, Hiragi H, San Gabriel MC, Yasuda N, Uno T. Isolation of canine parvovirus from a cat manifesting clinical signs of feline panleukopenia. *J Clin Microbiol.* 1996;34(9):2101–2105.
24. Mylonakis ME, Kalli I, Rallis TS. Canine parvoviral enteritis: an update on the clinical diagnosis, treatment, and prevention. *Vet Med Res Rep.* 2016;7:91–100
25. Orozco MM, Ceballos LA, M.C. P, Gürtler RE. Local threats and potential infectious hazards to maned wolves (*Chrysocyon brachyurus*) in the south-eastern Argentine Chaco. *Mammalia.* 2013;78(3):339–349.
26. Orozco MM, Miccio L, Enriquez GF, Iribarren F, Gürtler RE. Serologic evidence of canine parvovirus in domestic dogs, wild carnivores and marsupials in the humid Argentinean Chaco. *J Zoo Wildl Med.* 2014;45(3):555–563.
27. Parrish CR. Emergence, natural history, and variation of canine, mink, and feline parvoviruses. *Adv Virus Res.* 1990;38:403–450.
28. Parrish CR. Host range relationships and the evolution of canine parvovirus, *Vet Microbiol.* 1999;69(1–2):29–40.
29. Parrish CR, Kawaoka Y. The origins of new pandemic viruses: the acquisition of new host ranges by canine parvovirus and influenza A viruses. *Annu Rev Microbiol.* 2005; 59:553–586.
30. Parrish CR, O’Connell PH, Evermann JF, Carmichael LE. Natural variation of canine parvovirus. *Science.* 1985; 230(4729):1046–1048.
31. Steinel A, Parrish CR, Bloom ME, Truyen U. Parvovirus infections in wild carnivores. *J Wildl Dis.* 2001;37(3):594–607.
32. Veir JK. Canine parvoviral enteritis. In: Bonagura JD, Twedt DC (eds.). *Kirk’s current veterinary therapy XV.* 15th ed. St Louis (MO): Elsevier; 2014. p. 533–536.
33. Vieira FV, Hoffmann DJ, Fabri CUF, Bresciani KDS, Gameiro R, Flores EF, Cardoso TC. Circulation of canine parvovirus among dogs living in human-wildlife interface in the Atlantic Forest biome, Brazil. *Heliyon.* 2017;3(12):e00491.
34. World Wildlife Fund. WWF living forests reports: Chapter 5: saving forests at risk. [Internet]. 2015. [cited 2018 April 23]. Available from <http://www.wwf.de/fileadmin/fm-wwf/Publikationen-PDF/WWF-Living-Forests-Report-Chapter-5.pdf>

*Accepted for publication 18 September 2018*