Frequency and variation of canine parvovirus circulating in rural and wild carnivores in Northeastern Iran

Namroodi S.[1]*; Staji H.[2]

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Abstract

Canine parvovirus type 2 (CPV-2) affects many species of carnivores. There is no CPV vaccination program for rural dogs that roam in high densities around the villages and the edges of the wild ecosystem in Golestan Province, Northeastern Iran. We analyzed CPV infection in rural and wild carnivores of Golestan Province. Fecal samples from 69 road-killed wild animals, including 50 golden jackals (*Canis aureus*), 3 red foxes (*Vulpes vulpes*), 4 wildcats (*Felis silvestris*), 7 jungle cats (*Felis chaus*), and 4 Persian leopards (*Panthera*), as well as 55 rural dogs (*Canis lupus*), were analyzed via genomic DNA extraction, triplex PCR, and electrophoretic identification of CPV subtypes. We detected CPV in 13 (26%) of 50 sampled golden jackals and 18 (33%) of 55 rural dogs. The original CPV-2 genotype was not detected in any samples, but we identified CPV-2a in 4 golden jackals and 5 dogs, and CPV-2b in 9 jackals and 13 dogs. There was no statistically significant difference in CPV infection prevalence between male and female canids ($p>0.05$). CPV infection varied seasonally, with higher prevalence in cold seasons than in spring and summer ($p\leq0.05$). The prevalence of CPV infection was higher in younger canids than in older ones ($p\leq0.05$).

CPV infection in rural dogs and golden jackals highlights the presence of CPV in wild and rural ecosystems of the sampled areas, the necessity for the development and administration of a suitable vaccine for rural dogs, for continued research on CPV outbreak prevention, and for the development of rapid diagnostics.

Keywords: PCR, CPV-2a, CPV-2b, Canine parvovirus, Wild animals, Golestan Province.

1- Department of Environmental sciences, Faculty of fisheries and environmental Sciences, Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Iran

2- Department of Pathobiology, Faculty of Veterinary Medicine, Semnan University, Semnan, Iran

*Corresponding author's Email: namroodi@gau.ac.ir

Introduction

In recent decades infectious diseases have been described as a significant risk to many carnivores, including several endangered species. Canine parvovirus type 2 (CPV-2), family *Parvoviridae*, species *Carnivore protoparvo virus* is one of the most environmentally persistent and dangerous viruses affecting domestic and some wild species of carnivores. The virus causes high mortality in pups <6 months old and has also become a serious health problem in adult dogs (Parrish and Sykes, 2021). After its first identification in 1976 from a possible wild-carnivore origin, CPV-2 quickly acquired a global distribution (Carmichael, 2005).

Twenty years after the virus was identified, a mutation in the capsid gene resulted in the emergence of three antigenic variants: CPV-2a, CPV-2b, and CPV-2c (Nandi *et al*., 2019). The nucleotides critical for determining the virus antigenic type are located in the VP2 capsid protein encoding gene. The new viral variants are spreading among carnivore species and now infect domestic cats (*Felis catus*), cheetahs (*Acinonyx jubatus*), and Siberian tigers (*Panthera tigris altaica*) (Truyen, 1996: Steinel *et al*., 2000; Nandi *et al*., 2019). The predominant variants of CPV vary both spatially and temporally and the varying geographic distribution of viral variants emphasizes the importance of molecular epidemiologic studies.

The moderate, humid climate of Golestan Province, in Northeastern Iran, makes it favorable for many species of wild and domestic carnivores, as well as for CPV(Yelghei *et al*., 2020, Parrish and Sykes, 2021). Two carnivore species in Golestan Province, the Persian leopard (*Panthera pardus*) and the red fox (*Vulpes vulpes*) have been identified as endangered and least concern species, respectively by the IUCN (Yusefi *et al*., 2019). Data about CPV variants and their frequencies in rural areas near wild ecosystems may provide useful information to managers for designing and implementing CPV infection-control programs.

Golestan Province contains numerous villages adjoining wild ecosystems providing frequent opportunities for unvaccinated rural dogs to contact wild carnivores, potentially transmitting CPV. To gather information on the transmission, prevalence, identity of CPV strains, and carnivore species involved, we surveyed wild and rural carnivores for CPV variants using molecular methods.

Materials and methods

Fecal samples were obtained by sterilized swabs from road-killed wild carnivores and rural dogs in Golestan Province (Iran). The animals were from different carnivore species including 50 golden jackals (*Canis aureus*), 3 red foxes (*Vulpes vulpes*), 55 rural dogs (*Canis lupus familaris*), 4 wildcats (*Felis silvestris*), 7 jungle cats (*Felis chaus*), and 4 Persian leopards (*Panthera pardus*). There were no exclusion criteria set for this investigation and therefore all the collected specimens were assessed. Fecal samples from each

animal was preserved in -20˚C prior to molecular examination. Genomic extraction from fecal samples was performed by using a commercial DNA extraction kit (Bioneer, Daejeon, South Korea) according to the manufacturer's instructions. The DNA concentration and purity were measured using a Nanodrop spectrophotometer (SmartNano, Canada) and were stored at −20°C until use.

Three sets of primers were used (Table 1) in a triplex-PCR to amplify the target regions for detection and confirmation of CPV infection in fecal specimens (Mizak and Rzezutka, 1999; Abedi *et al*., 2018). Amplifications of DNA fragments were carried out as following reagent conditions: The amplification reaction was performed in 25 µL containing 1 µM of each primer, 4 µL of extracted total DNA of each sample as template, 4 µL of distilled water and 13 µL of a commercial Master mix (Cinnagen,Tehran, Iran). The cycling program was as follows: initially, 1 min at 94˚C for denaturation, followed by 30 cycles of 94˚C for for 45 s, at 49°C for 50 s and 72°C for 1 min and a final elongation step at 72°C for 5 min. The amplified DNA fragments were electrophoresed for 90 min at 60 V through 1.2% agarose gels in 1X TBE buffer and stained with ethidium bromide. The agarose gel was visualized under the UV transillumiator (Nanolytic, England) followed by electrophoresis step. Finally, fragments size was determined by comparison with a Mid-Range DNA ladder.

Table 1: The sequences of used primers.

Gene	Sequence (5´⟶3´)	Product size (bp)
CPV-2b	(f): CTTTAACCTTCCTGTAACAG (r): CATAGTTAAATTGGTTATCTAC	427
CPV-2a	(f): GAAGAGTGGTTGTAAATAATA (r): CCTATATCACCAAAGTTAGTAG	681
CPV 2	(f): AGCTATGAGATCTGAGACAT (r): AGTATGTTAATATAATTTTCTAGGTGC	1198

The statistical significance of differences in frequencies of CPV types among carnivore species in different seasons and between male and female animals was evaluated using chi-square tests on contingency tables and Fisher's exact tests with a significance level of P = 0.05, using SPSS (version 21) software.

Results

We used the finding of CPV DNA in feces as our marker of infection. We detected CPV DNA in 13 (26%) of the 50 sampled golden jackals and 18 (33%) of the 55 rural dogs but none of the sampled red foxes, wildcats, jungle cats, or Persian leopards (Table 2).

The original CPV-2 genotype was not detected in any samples but we

identified CPV-2a in 4 golden jackals and 5 dogs and CPV-2b in 9 jackals and 13 dogs. There was no significant difference between male and female canids in the prevalence of CPV infection. CPV infection in sampled canids showed different trends in warm (spring and summer) and cold seasons (winter and autumn) with higher prevalence in cold seasons. Higher CPV infection prevalence was detected in younger canids than in older canids (Table 3).

Table 2: Canine parvovirus (CPV) infection frequencies in sampled carnivores by species.

Family	Sampled species	CPV prevalence (%)
Canidae	*Canis aureus*=50	13/50 (26%)
	Vulpes vulpes=3	0/3
	Canis lupus familiaris=55	18/55 (33%)
Felidae	*Felis silvestris*=4	0
	Felis chaus=7	0
	Panthera pardus=4	0

Table 3:. Canine parvovirus (CPV) infection frequencies in sampled golden jackals and rural dogs by season, sex, and age class. Pos = Positive; D = dog; J = Jackal.

Surveyed factor	Season: Pos/tested (%)	Sex: Pos/tested (%)	Age: Pos/tested (%)
CPV infection frequency	Spring: J =1/8(12.5%), D=1/4 (25%) Summer: J =1/7 (14%), D= 2/8 (25%) Autumn: J =5/15(33%), D= 7/19 (37%) Winter: J =6/20 (30%), D= 8/24 (33%)	Male: D=12/37(32%) J=9/34 (26%) Female: D= 6/18 (33%) J=4/16 (25%)	1-2 years old: D=10/20 (50%) J=9/15 (60%) 2-3 years old: D=4/15 (27%) J=2/20 (10%) 4-5 years old: D=4/20 (20%) J=2/15 (13%)

Discussion

The first detection of CPV-2 in Iran, was in a 7-month-old male dog from Tehran (Hemmatzadeh and Jamshidi, 2002). Since that discovery, CPV infection has continued to be documented in various areas of Iran. In a survey on fecal samples of 55 dogs (50 with acute hemorrhagic diarrhea syndrome and 5 healthy) in two age groups (<6 months and >6 months) in Ahvaz Province, southwestern Iran, Mosallanejad *et al*. (2008) found 17% prevalence of CPV infection (Mosallanejad *et al*., 2008). Dastmalchi Saei *et al*. (2017) found CPV in 10 of 35 (29%) dogs (19 diarrheic and 16 healthy) from Urmia, in western Iran where the climate is colder and drier than in Golestan Province (Dastmalchi Saei *et al*., 2017).

Finally, in Khuzestan Province, Vakili *et al*. (2016) tested 50 hemorrhagic diarrheic domestic dogs by immunochromatography and PCR and detected CPV-2 antigen and nucleic acid in 33 and 50 dogs, respectively (Vakili *et al*., 2016). There are similar studies in other parts of the world. Testing dogs in different regions of China, Xu *et al*. (2015) found 46.6% CPV prevalence

and Yi *et al*. (2016) found 56% prevalence (Xu *et al*., 2015; Yi *et al*., 2016) Using molecular surveys, Clegg et. al. (2011) documented CPV infection in 62 of 97 (64%) dog fecal samples in the UK (Clegg *et al*., 2011).

Most reports mentioned herein are based on epidemiologic studies on domestic dogs. Studies of CPV infection frequency and CPV variants circulating in wild carnivores and cats are very limited. Namroodi *et al* (2017) reported 24% CPV infection of road-killed golden jackals in Golestan Province. 630 fecal samples of jackals have been surveyed for presence of CPV in Turkey and only %3 of them were CPV infected (Kurucay *et al*., 2023). There is no other similar study on CPV frequency and variants circulating in jackals; the only related data are about CPV antibody frequency in Kenya, Zimbabwe, and Israel (Alexander *et al*., 1994; Spencer *et al*., 1999; Shamir *et al*., 2001).

However, some investigators have studied the epidemiology of CPV in other wild carnivores. In a study on CPV infection in the gray wolf population of Superior National Forest, Minnesota, USA, only two of 206 feces samples were positive by real-time PCR. (Mech *et al*., 2008) In a similar molecular study of 227 free-ranging wild carnivores of 12 species in Portugal, CPV was detected in only three gray wolves and one stone marten (*Martes foina*) (Miranda *et al*., 2017). In a study in Misiones Province, Argentina, Orozco *et al*. (2018) detected no CPV shedding by PCR in feces samples from 115 wild carnivores of five species (Orozco *et al*., 2018). CPV infection was detected in 38% of 20 sampled coyotes in Brazil (Spera *et al*., 2020).

It seems that our failure to detect CPV infection in felids was largely due to the very small sample sizes of felid species tested (Table 1). Additional studies with greater sample sizes are needed. From comparison of percent of CPV infection of rural dogs (33%) and golden jackals (26%) in current study with other similar studies from Iran and other part of the world, high contamination of Golestan Province with CPV can be concluded.
Differences in CPV prevalence found in similar studies may be due to numerous factors in the study areas, including the population densities of canids, annual mean temperature, maximum temperature of the warmest month, annual precipitation, as well as sensitivity of the detection method (Millán *et al*. 2016).

Besides the difference in CPV infection frequency among canid populations, the geographic distribution of CPV strains differs among regions of the world. In China, Korea, India, Argentina, and some European countries, CPV-2a has been introduced as the dominant CPV variant in the domestic dog population (Battilani *et al*., 2001; Decaro *et al*., 2007; Zhong *et al*., 2014; Jeoung *et al*., 2008; Raj *et al*., 2010; Calderón *et al*., 2011). But in Brazil, Taiwan, Japan, and Switzerland, CPV-2b has been identified in a higher proportion of cases than CPV-2a and CPV-2c (Pereira *et al*., 2000, Wang *et al*., 2005, Doki *et al*., 2006, Truyen *et al*., 2000). Yet, new epidemiologic

research shows that CPV-2c is the most prevalent variant in Germany, Spain, and Italy (Decaro *et al*., 2007, Decaro *et al*., 2011).

One of the most comprehensive studies of wild carnivores was conducted on the island of Newfoundland, Canada. From 85 coyotes (*Canis latrans*), 22 red foxes (*Vulpes vulpes deletrix*) and 38 lynx (*Lynx Canadensis subsolanus*), only two strains of CPV were detected, (tow CPV-2b and one CPV-2a), all from coyotes (Canuti *et al*., 2017). In a 10-year molecular study of 19 domestic cats and 57 hunted wild carnivores in Bulgaria, only one cat, one gray wolf, and one red fox were infected with CPV-2a (Filipov *et al*., 2016). In a molecular study on wild free ranging carnivores of Portugal, CPV-2a and CPV-2c were detected in three gray wolves and a stone marten, respectively (Miranda *et al*., 2017).

In Iran, molecular studies by Firoozjaii *et al*. (2011), Dastmalchi *et al*. (2017), Vakili *et al*. (2016), and Mohiyaddin *et al*. (2013) showed that the prevalent antigenic type in domestic dog populations was type CPV-2b and, to a lesser extent, CPV-2a (Firoozjaii *et al*., 2011;Mohyedini *et al*. 2013; Vakili *et al*. 2016; Dastmalchi Saei *et al*., 2017).

But current survey of Faraji *et al*. (2021) on 206 CPV suspected dogs of different regions of Iran showed that CPV-2a is the most predominant strain in Iran. However, our results in rural and wild ecosystems in Golestan Province appear not to confirm results (Faraji *et al*., 2021).

Failure to detect CPV-2 in sampled feces of current study combined with other studies in Iran, support the theory of complete replacement of CPV-2 by a new variant (Martella *et al*., 2005). Previous studies have revealed that some species of wild carnivores can play a role in the evolution of CPV-2 into a new variant. This possibility emphasizes the importance of further study of wild carnivore species of Golestan Province and their relationship to CPV.

In Canada, the seasonal incidence of CPV infection in domestic dogs was reported to be higher in the summer months when there are more newborn, susceptible pups (Houston *et al*., 1996). This contrasts with our finding of higher prevalence of CPV infection in autumn and winter. In our case, sampling of canids >1 yr old may have led to that result. To clarify this discrepancy, an Iranian study with a larger sample size, containing younger individuals, and conducted in in warmer as well as well as colder seasons will be needed.

Our failure to detect a significant difference in CPV infection between male and female rural dogs or jackals agrees with findings of previous serologic surveys of canids (Mech *et al*., 2008; Mosallanejad *et al*., 2008). From such a result it can be concluded that sexual behavior does not affect CPV infection in surveyed canids species but it should be kept in mind that the numbers of surveyed canids in this study was limited and more extensive researches are needed to can certainly conclude this theory.

Finding of more frequent CPV infection in younger, than in older canids in current study has been observed previously, and was explained by a higher sensitivity to CPV infection in younger animals (Miranda *et al*., 2015). Nevertheless, others have detected no difference in CPV infection in rural dogs in different age classes (Mosallanejad *et al*., 2008).

A higher prevalence of CPV infection in rural dogs than in golden jackals in current study might be due to the lower contact rates between wild canids compared to rural dogs (Hoelzer and Parrish, 2010). Because of the sparsity of studies, the impacts of CPV infection on Iranian wild carnivores are largely unknown. Nevertheless, our results revealed that CPV can be a major factor influencing wild carnivore populations in Golestan Province, especially given the favorable environmental conditions for harboring such a persistent virus. Currently, there is no CPV-2 vaccination program for rural dogs in Golestan Province. Research toward the identification of CPV strains circulating in wild carnivores and domestic dogs, and the development and application of an appropriate vaccine strain for domestic dogs are critical for preventing future CPV infection outbreaks in wild and domestic canids in Golestan Province.

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Conflict of interest

There is no conflict of interest between the authors.

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