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## Serologic Survey for Canine Distemper Virus and Canine Parvovirus in Free-ranging Wild Carnivores from Portugal

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**ABSTRACT:** A serologic survey for *Canine distemper virus* (CDV) and canine parvovirus (CPV) was performed on serum and lung extract from an opportunistic sample of 120 free-ranging wild carnivores (13 species) from Portugal, collected from 1995 to 2006. Antibodies to CDV were detected in wolf (*Canis lupus*; 3/27) and red fox (*Vulpes vulpes*; 2/22). Antibodies to CPV were detected in wolf (9/28), red fox (2/14), wildcat (*Felis silvestris*; 1/8), genet (*Genetta genetta*; 17/18), and stone marten (*Martes foina*; 3/17). Antibodies to CPV were detected throughout the study, whereas for CDV antibodies were detected in 3 of 10 yr and only during winter. The extremely high CPV antibody prevalence in genets is unprecedented. Although based on a limited sample, these data suggest widespread exposure of free-ranging Iberian carnivores to CDV and CPV.

**Key words:** *Canine distemper virus*, canine parvovirus, Portugal, serologic survey, wild carnivores.

Carnivore population declines have been associated with several infectious diseases (Funk et al., 2001), emphasizing the significance of pathogens in the population dynamics of these species. Two important pathogens that can affect conservation efforts for wild carnivores are *Canine distemper virus* (CDV; *Morbillivirus*, *Paramyxoviridae*) and canine parvovirus (CPV; isolate of *Feline panleukopenia virus*; *Parvovirus*, *Parvoviridae*, Funk et al., 2001). Although numerous wild species in the Canidae, Felidae, Mustelidae, and Viverridae are susceptible to CDV, the epidemiology of this disease in free-ranging carnivores is poorly understood (Funk et al., 2001, Williams, 2001). Several parvoviruses infect wild carnivores, including CPV, *Feline panleukopenia virus* (FPLV), and the antigenically distinct *Aleutian mink disease virus* (ADV;

Steinel et al., 2001). Both CPV and FPLV cause disease characterized by gastrointestinal symptoms but can also cause neurologic and cardiac manifestations in juveniles (Steinel et al., 2001). Infection with CPV and FPLV has been demonstrated in Canidae, Felidae, and Mustelidae and has been suggested in Viverridae (Barker and Parrish, 2001, Steinel et al., 2001).

There are several conservation efforts directed at regional carnivore populations, where infectious diseases could have a significant effect. Northern Portugal is inhabited by approximately 60 wolf (*Canis lupus*) packs that are connected to the larger Spanish population. In central Portugal, there are six to nine packs that have been demographically isolated for several decades (Pimenta et al., 2005). This isolated population is very unstable, and successful reproduction is seldom recorded (Pimenta et al., 2005). Although infectious diseases could represent a significant threat to the survival of this population, the status of even common diseases in this population is unknown. In addition, the Iberian lynx (*Lynx pardinus*) is nearly, if not, extinct in Portugal, and captive breeding is taking place with the aim of reintroducing the species to former range. Although an infectious disease risk assessment is needed to guide reintroduction efforts (Sarmiento et al., 2005), data on disease prevalence for potential sites for introduction are lacking. This study aims to obtain baseline prevalence estimates for antibodies to CDV and CPV in Portuguese carnivore species.

The study was conducted on 120 free-ranging carnivores sampled from 1995 to 2006; either serum ( $n=18$ ) or lung tissue

extract (LTE,  $n=105$ ) were collected from these animals. Blood was collected from both live-captured and dead (hunted or found recently dead) animals and allowed to clot. Clotted blood was centrifuged and serum frozen at  $-20$  C. Dead carnivores were subjected to a standard necropsy procedure, and a portion of the lungs was collected and frozen at  $-20$  C as part of the collection of Banco de Tecidos de Vertebrados Selvagens (BTVS/ICNB) and Sistema de Monitorização de Lobos Mortos (SMLM/ICNB). Lung samples were thawed, and LTE was obtained as described (Ferroglio et al., 2000), except that approximately 2.5 g of tissue was used. Preservation state of the carcass (fresh or decomposed, according to gross inspection), date, and site of collection were recorded. From one wolf, one red fox (*Vulpes vulpes*), and one genet (*Genetta genetta*), we obtained paired serum and LTE samples. The study area was continental Portugal ( $42^{\circ}9'15''-36^{\circ}57'42''$ N,  $9^{\circ}30'2''-6^{\circ}11'23''$ W).

A microneutralization test (Appel and Robson, 1973) was used for CDV antibody testing. Serial twofold dilutions of samples were incubated in 96-well flat-bottom plates for 1 hr at 37 C with 10–30 median tissue culture infective doses of Onder-spoot strain of CDV. Vero cells, in Minimum Essential Medium supplemented with penicillin/streptomycin and 10% fetal bovine serum, were added to a final concentration of  $2 \times 10^4$  cells per well. After incubation for 4 days, the extent of neutralization was evaluated, and titers were expressed as the reciprocal of the highest serum dilution that fully suppressed the specific cytopathic effect. A titer greater than eight was considered positive. Samples were tested twice, with four replicates in each test.

A hemagglutination inhibition test (Carmichael et al., 1980) was used to test for CPV antibodies. Briefly, samples were heat-inactivated and absorbed with porcine erythrocytes to remove nonspecific hemagglutinins. Four to eight units of

antigen (CPV, Cornell strain) were mixed with sample dilutions (twofold) and incubated for 1 hr at room temperature. A 0.5% suspension of washed erythrocytes (pig; *Sus scrofa*) was then added, and the test was read after incubation at 4 C for 2 h. A titer greater than 40 was considered positive.

Antibodies against CDV were detected in five canids (three wolves and two red foxes). Antibodies against CPV were found in species in Canidae (nine wolves, two red foxes), Felidae (one wildcat, *Felis silvestris*), Viverridae (17 genets), and Mustelidae (three stone martens, *Martes foina*; Table 1). Positive antibody titers for CDV were 32–64 in wolf and 512 in red fox. Positive CPV antibody titers were 80–320 in canids and stone marten, 160 in the wildcat, and 80–1,280 in genet.

Canine parvovirus antibodies were detected in nine of 20 wolves from the northern population; this prevalence was significantly higher than that observed in central Portugal (0/7,  $\chi^2=5.3$ ,  $P=0.02$ , 1 df). The CPV-seropositive wildcat originated from a stable population in southern Portugal.

The temporal pattern of detectable antibodies for both viruses was analyzed in the Canidae. Antibodies to CDV were detected in one wolf in 1995 and 1999 (from different locations) and in one wolf and two red foxes, collected 8 days and 50 km apart in 2002; antibodies to CPV were detected in 8 of 10 yr. All CDV-antibody-positive animals were collected in December, January, and February, whereas CPV-antibody-positive animals were detected in every season, except summer.

The three animals with paired serum and LTE samples yielded the same test results for both samples: canids were negative for CPV, with antibody titers at least 1–2 times lower in LTE ( $<10$ ) compared with serum (10 and 20), whereas in the genet, they were identical (640) in both samples. *Canine distemper virus* serology was negative ( $<8$ ) in these samples.

TABLE 1. Results of the serologic survey for CDV (seroneutralization test) and CPV (hemagglutination inhibition test) in free-ranging wild carnivores from Portugal. Results are presented by species and taxonomic family.<sup>a</sup>

Family	Species	CDV			CPV		
		Positives/ total tested	% Positive	95% CI	Positives/total tested	% Positive	95% CI
<i>Canidae</i>	Wolf ( <i>Canis lupus</i> )	3/27	11.1	2.2–27.4	9/28	32.1	15.9–52.4
	Red fox ( <i>Vulpes vulpes</i> )	2/22	9.1	1.1–29.2	2/14	14.3	1.8–42.8
	Total	5/49	10.2	3.3–21.8	11/41	26.8	14.2–42.9
<i>Felidae</i>	Wild cat ( <i>Felis silvestris</i> )	0/8	0	0–36.9	1/8	12.5	0.3–52.7
	Total	0/8	0	0–36.9	1/8	12.5	0.3–52.7
<i>Viverridae</i>	Common genet ( <i>Genetta genetta</i> )	0/18	0	0–18.5	17/18	94.4	72.7–99.9
	Mongoose ( <i>Herpestes ichneumon</i> )	0/5	0	0–52.2	0/5	0	0–52.2
	Total	0/23	0	0–14.8	17/23	73.9	51.6–89.8
<i>Mustelidae</i>	Stone marten ( <i>Martes foina</i> )	0/17	0	0–19.5	3/17	17.6	3.8–43.4
	Pine marten ( <i>Martes martes</i> )	0/2	0	0–84.2	0/2	0	0–84.2
	European polecat ( <i>Mustela putorius</i> )	0/7	0	0–41.0	0/7	0	0–41.0
	Eurasian otter ( <i>Lutra lutra</i> )	0/5	0	0–52.2	0/5	0	0–52.2
	Eurasian badger ( <i>Meles meles</i> )	0/2	0	0–84.2	0/2	0	0–84.2
	American mink ( <i>Mustela vison</i> )	0/1	0	0–97.5	0/1	0	0–97.5
	Weasel ( <i>Mustela nivalis</i> )	0/2	0	0–84.2	0/2	0	0–84.2
	Stoat ( <i>Mustela erminea</i> )	0/1	0	0–97.5	0/1	0	0–97.5
	Total	0/37	0	0–9.5	3/37	8.1	1.7–21.4
	Total	5/117	4.3	0.6–8.0	32/109	29.4	20.8–38.0

<sup>a</sup> CDV = *Canine distemper virus*, CPV = canine parvovirus, 95% CI = 95% confidence interval.

Overall, 57 samples were obtained from fresh carcasses of animals, and 52 from decomposed carcasses; we had no data for 11 samples. Median CPV titers in LTE of seropositive genets were 160 for fresh carcasses ( $n=12$ ) and 240 for decomposed carcasses ( $n=4$ ); the difference is not statistically significant (Mann-Whitney  $U$ -test=24,  $P=1.00$ ). In wolves, median CPV titers were 160 for fresh carcasses ( $n=5$ ) and 80 for decomposed carcasses ( $n=4$ ); this difference is also not statistically significant ( $U=10$ ,  $P=1.00$ ).

Our results confirm previous exposures to morbillivirus and parvovirus in carnivore populations in Portugal, but the specific viruses responsible for these detected antibodies were not identified. The prevalence rate for CDV-antibodies in red fox was similar to antibody prevalence rates

reported from Central Europe (4–13%; Truyen et al., 1998, Damien et al., 2002) but lower than those from Spain (17%); antibody prevalence to CDV in wolf was also higher in Spain (24%; Sobrino et al., 2008).

Temporal pattern of prevalence of antibodies to CDV suggests that CDV is epidemic in wild canids, as reported in Spain (Sobrino et al., 2008). Relatively dense populations of susceptible hosts are usually needed to sustain CDV circulation (Williams, 2001); red fox can sometimes attain high densities, but this is not the case for wolf (Pimenta et al., 2005). On the other hand, domestic dogs are widespread, achieve high densities, and can maintain CDV and act as reservoirs of infection for wild carnivores. Transmission could occur through predation or scavenging of a

domestic carnivore carcass (Butler et al., 2004) because dogs are a small part of the diet of Portuguese wolves (Vos, 2000, Roque et al., 2001). This possible role of domestic carnivores on the epidemiologic cycle of wildlife CDV needs to be ascertained.

Wolf populations with low genetic variability can have impaired immune response, increasing their susceptibility to both CDV and CPV (Hedrick et al., 2003). There is some evidence that the remnant wolf population in central Portugal has low genetic variability (Godinho et al., 2005); interestingly, one possible explanation for the apparent absence of CPV antibodies in this population could be a high case-fatality rate. The interaction between genetic variability, immunologic competence, and CPV pathogenesis in this population needs to be investigated.

Although based on a small sample, our results suggest a low antibody prevalence for parvovirus in the wildcat. These results are consistent with results from other surveys (Leutenegger et al., 2002, Ostrowski et al., 2003) and confirm that wild felids can be exposed to parvoviruses (presumably FPLV) that inhabit potential reintroduction sites for the Iberian lynx (Sarmiento et al., 2005).

Regarding CPV, Truyen et al. (1998) and Frolich et al. (2005) reported an antibody prevalence similar to ours in both red fox (9–13%) and stone marten (17%) from Germany. In Spain, Sobrino et al. (2008) report a higher antibody prevalence for CPV in wolves (62%) but lower in red fox (5%).

There is little information on parvovirus (other than ADV) infection in wild viverrids; in captive animals, a parvovirus was detected in the feces of healthy genets in a zoo (Barker and Parrish, 2001), and Ikeda et al. (1999) reported four of four captive civets (*Paguma larvata taiwana*) as CPV-antibody positive in southeast Asia. In France, three of 68 wild genets were antibody positive for ADV (Fournier-Chambrillon et al., 2004), and this virus

was shown to occur in wild mustelids in Spain (Mañas et al., 2001). To our knowledge, the present study is the first to report a high antibody prevalence rate in free-ranging genets. These data, in the absence of similar antibody prevalence rates in other abundant sympatric carnivores with overlapping ecologic niches (Carvalho and Gomes, 2001), suggest the existence of a host-adapted virus. This should be the subject of further studies.

Antibody prevalence rates reported in this study may underestimate true prevalence because the use of LTE from decomposed carcasses might have reduced test sensitivity. Tryland et al. (2006) have shown that antibodies can be detected in body fluids from red fox carcasses up to 11 days postmortem, albeit at decreasing titers. This is consistent with our observation that CPV titers did not vary significantly with carcass putrefaction in genet and wolf. Tryland et al. (2006) deemed material from decomposed fox carcasses valuable for serologic testing.

Test sensitivity related to lung extract and serum have been compared, with sensitivity estimates ranging 22% to 100%, depending on the host-pathogen system (e.g., Morner et al., 1988, Ferroglio et al., 2000). Our results for paired LTE and serum samples suggest that antibody titers were identical or reduced by two dilutions, as reported by Ferroglio et al. (2000). If this is the case, the decrease in sensitivity because of the use of LTE should be limited and would only affect results from animals with low antibody titers.

This study demonstrates extensive exposure of free-ranging Iberian wild carnivores to both morbilliviruses and parvoviruses in Portugal; this is especially true with genets, where a high prevalence of antibodies to parvovirus was detected. Further efforts to isolate and characterize these viruses are warranted. Larger-sampled surveys and longitudinal studies are needed to understand the epidemiology

and possible impacts of these diseases on the conservation of Iberian carnivores.

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