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Prevalence of antibodies against canine distemper virus and canine parvovirus among foxes and wolves from Spain.

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ABSTRACT

Viral diseases can influence the population dynamics of wild carnivores and can have effects on carnivore conservation. Hence, a serologic survey was conducted in an opportunistic sample of 137 foxes (*Vulpes vulpes*) and 37 wolves (*Canis lupus*) in Spain for 1997-2007 to detect antibodies against canine distemper virus (CDV) and against canine parvovirus (CPV) by indirect ELISA. Antibodies against CDV were detected in 18.7% of the analyzed animals and antibodies against CPV in 17.2%. There was no difference in antibody prevalence to CDV between both species, even in the same region ($P>0.05$), but there was a significant difference in antibody prevalence to CPV between foxes (5.1%) and wolves (62.2%) ($P<0.05$). In fox populations there was a significant difference in antibody prevalence to CDV between geographic areas (Aragón 26.4%, La Mancha 7.8%, $P<0.05$). In wolf populations there was a significantly higher antibody prevalence against CPV ($P<0.05$) in Castilla y León (100%) than in the Cantabric region (53.3%). There was no significant sex or age related difference in the antibody prevalence against CDV or CPV in foxes. These results indicate that contact with CDV is widespread among wild canid populations in Spain and that CPV is endemic in the Iberian wolf population. The implications of these results are briefly discussed.

Keywords: *Canis lupus*, Serosurvey, Spain, *Vulpes vulpes*, Wild canids.

Introduction

Canine distemper virus (CDV) and canine parvovirus (CPV) are common pathogens of domestic and wild carnivores and have a worldwide distribution. CDV is a Morbillivirus (family *Paramyxoviridae*) that is very resistant to cool temperatures but quickly inactivated by ultraviolet light and by heat and drying. It is transmitted by aerosols or contact with oral, respiratory, and ocular fluids and exudates containing the virus. Therefore, dense populations of susceptible animals are needed to sustain epidemics (Williams and Barker, 2001). CDV affects species belonging to all families of the order carnivora and seems to have the major impact in wild carnivores and in captivity (Montali et al., 1987). It is known that CDV caused the disappearance of the last wild population of black-footed ferret (*Mustela nigripes*) (Thorne and Williams, 1988), and has also been considered responsible of declines of endangered species such as African wild dog (*Lycaon pictus*) during the epizootic in domestic dogs (*Canis familiaris*) and wild carnivores in the Serengeti (Alexander and Appel, 1994). In Spain, CDV has been identified as cause of death or disease in domestic dogs (Nieto et al., 1987), polecats (*Mustela putorius*), American mink (*Mustela vison*), genet (*Genetta genetta*), fox (*Vulpes vulpes*) and stone marten (*Martes foina*) (López-Peña et al., 2001). In contrast, CPV is a Parvovirus (family *Parvoviridae*) that is very hardy, able to survive up to 6 months at room temperature. It is transmited by the fecal-oral route, probably mainly through ingestion of virus from the environment, rather than by direct contact with infected animals (Williams and Barker, 2001). CPV has a more limited host range affecting different canids (Parrish, 1990). Although CPV has been linked with mortality in young wolves (Johnson et al., 1994) and coyotes (Gese et al., 1997)

and could threaten the viability of small isolated populations (Mech and Goyal, 1993, 1995), its impact in wild canid populations is largely unknown. In Europe, the presence of the virus has been reported in wild canid populations in Italy (Martinello et al., 1997) and data on strains isolated from wolves demonstrated that the same strain of CPV can circulate among domestic and wild canids (Battilani et al., 2001). No mortality due to CPV has been reported in wild canids from Spain but CPV is common in domestic dogs (Decaro et al., 2006).

The Iberian wolf (*Canis lupus signatus*) is considered a vulnerable species and its population is estimated at a minimum of 2000 individuals (Blanco, 1998). Although the main factors that can affect its survival are human causes or prey availability, infectious diseases can also act as a mortality source. In fact, wolf mortality due to CDV and CPV has been reported (Carbyn, 1982; Mech et al., 1997). The fox (*Vulpes vulpes*) in contrast, is an abundant species with a wide distribution in the Spanish mainland (Blanco, 1998). This species is susceptible to a number of diseases including CDV and CPV (Artois et al., 1996). Thus, it could be a source of infection to other less abundant species that live sympatrically. Additionally, feral or free roaming domestic dogs may also become a source of infections for wild canids (Alexander and Appel, 1994). However, feral dog abundances in Spain are low as compared to fox abundances (The authors, unpublished data).

The objective in this study was to determine the prevalence of serum antibodies to CDV and CPV in Spanish foxes and wolves, and their differences across age and sex classes and geographical regions.

Materials and methods

Sampling

In the period of 1997-2007, serum samples were collected from 37 wolf and 137 fox carcasses from 4 Spanish regions including the Cantabric coast, Castilla y León, Aragón and La Mancha (Figure 1). All animals were legally obtained (road kills and some foxes hunted for population control).

The serum samples were obtained by cardiac puncture, centrifuged, and stored at -20ºC until their analysis. Age class (yearling <1 year vs. adult >1 year) was determined by tooth eruption and the degree of tooth wear (Sáenz de Buruaga et al., 2001). As a consecuence of opportunistic sampling, the age or sex was not known for 26 foxes and 25 wolves.

Serum antibody testing

Antibodies to CDV and CPV were determined by indirect enzyme-linked inmunoassay (ELISA), using commercial kits and following the manufacturer's instructions (Ingenasa, Madrid, Spain) (Corrain et al., 2007). To test for CDV, the serum samples were diluted 1/100, and anti-dog IgG was used as conjugate. Samples were considered positive if OD (optic density) value was higher than absorbance of positive control * 0.2. To test for CPV with the same dilution and conjugate, samples were considered positive if the ratio S/P (sample optic density / positive control optic density) was ≥ 0.15.

Statistics

Seroprevalence was statistically analyzed considering the variables geographical area, sex, age and host species using the SPSS 14.0 software. We used Chi-square tests and Fisher test, with a 95% confidence level and a P value <0.05 was considered significant.

Results

Total antibody prevalence against CDV was 18.7%, being positive 9 (24.3%) of 37 wolves and 23 (17.1 %) of 134 foxes. Total antibody prevalence against CPV was 17.2% being positive 23 (62.2%) of 37 wolves and 7 (5.1%) of 137 foxes. There was no significant difference in the prevalence of antibodies against CDV between both species ($P>0.05$), but there was a significant difference in antibodies against CPV ($P<0.05$).

The antibody prevalence distribution against CDV and CPV by sex and age is shown in Table 1. Although there was no significant difference between sex and age classes in anti-CDV antibody prevalence in foxes, there was a slightly higher prevalence in adults than in juveniles ($P=0.1$). There was no significant difference by age or sex in antibodies against CPV. Differences in antibody prevalence by age or sex were not analyzed in wolves since these variables were unknown for most animals.

The antibody prevalence against CDV and CPV by regions is presented in Figure 1. In foxes, there was a significant difference between Aragón (26.4%) and La Mancha (7.8%) ($P<0.05$), but not with other regions ($P>0.05$). In wolves, there was a significant difference in CPV prevalence between the Cantabric region (53.3%) and Castilla y León (100%) ($P<0.05$). In the Cantabric region, where both fox and wolf sera were available, the prevalence of antibodies against CDV and CPV was of 22.2% and 23.3% and 10.6% and 53.3% for foxes and wolves respectively (Figure 1). There was no difference in CDV prevalence between both species in this area ($P>0.05$), but there was a significant difference between both species in antibody prevalence against CPV.

Discussion

This is the first report of contact with CPV and CDV in the wolf in Spain. Various tests are available to detect antibodies to CDV and CPV. The standard technique for CDV is the virus neutralization (Williams and Barker et al., 2001), but it is expensive, time-

126 consuming, requires specialised laboratory facilities and good quality sera, with little or
127 no haemolysis. Hence, we decided not to use this tool to analyze our serum samples
128 (most of them from dead-found animals and with haemolysis). Recently, the ELISA
129 tests have been shown to be sensitive and specific against CDV and CPV (Ohashi et al.,
130 2001; Phukan et al., 2005).
131 The prevalence to CDV in wolves in our study (24.3%) was similar to the prevalence
132 described for this species in North America (7-67 %, Stephenson et al., 1982; Johnson
133 et al. 1994, Philippa et al., 2004). In contrast, the prevalence in foxes was 17.1%, which
134 is a higher value as compared to previous results for this species in central Europe (5-
135 13%, Frölich et al. 2000, Damien et al., 2002) and in North America (11%, Amundson
136 and Yuill 1981). However, the different serological tools used in some cases make the
137 interpretation and comparison between studies difficult.
138 The prevalence of antibodies against CDV was not age-specific or sex-specific in foxes.
139 The regional differences in CDV antibody prevalence could be due to different fox
140 densities, different spatial aggregation, or different degree of contact with domestic
141 dogs (e.g. Gortázar et al. 2003). In regions such as La Mancha, where foxes apparently
142 had less contact with CDV, the introduction of this pathogen could cause an epidemic
143 outbreak, because most individuals would be immunologically naïve (Appel, 1987).
144 This would have conservation implications since eventually CDV could spread from the
145 abundant fox population to other sympatric carnivores and affect endangered species
146 such as the lynx. Epidemic distemper outbreaks have happened in La Mancha in 1993
147 causing a 70% decrease in fox relative abundance (dropping counts, Ramos, 1995) and
148 in North-west Spain in 1997 (Marta Muñoz, pers. comm.).
149 The relatively high prevalence of antibodies against CPV in the Iberian wolf (62.2%), is
150 within the range reported for this species in North America (13-95%, Zarnke and

Ballard 1987; Mech and Goyal, 1993). This high prevalence suggests a high exposure to infection but does not inform about disease, because the prevalence is measured in surviving individuals (Arjo et al., 2003). The prevalence of antibodies against CPV in wolves was significantly lower in the Cantabric region (53.3%) than in Castilla y León (100%). Since no other practical way of sampling wolves was available, our sampling strategy reduced all the possible inferences to the whole population.

The anti-CPV antibody prevalence in foxes (5.1%) was within the range reported by other authors in Europe (0-9%, Mulley et al., 1982, Frölich et al., 2005) although different tools were used for serological testing. The higher prevalence against CPV in wolves than in sympatric foxes is surprising and suggests that foxes are not an important source of infection to wolves. An alternative explanation could be that CPV does not affect foxes. In an experimental infection of CPV in foxes Barker et al. (1983) demonstrated the resistance of the species to the disease. Also, Truyen et al. (1998) amplified DNA sequences from tissues of free-ranging foxes and compared them with the prototype viruses from dogs and cats. The parvovirus sequence was indicative of a true intermediate between CPV and feline panleucopenia virus, representing a link between those viral groups.

We conclude that foxes and wolves from Spain have contact with CDV and that CPV is endemic in Iberian wolf populations. This information is of use in the frame of carnivore conservation. Further investigations are needed to study the epidemiology of these viral agents in the wild canid populations.

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271

272 Table 1. Distribution of antibodies against CDV and CPV by sex and age in foxes and
273 wolves.

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277 Table 1.

	CDV antibody prevalence				CPV antibody prevalence			
	Age		Sex		Age		Sex	
	Adults	<1 year	Males	Females	Adults	<1 year	Males	Females
Fox	15/66 (22.7)[a]	4/45 (8.9)	14/63 (22.2)	5/52 (9.6)	1/60 (1.7)	2/52 (3.8)	2/61 (3.3)	3/55 (5.5)
Wolf	4/8 (50)	0/4 (0)	1/3 (33.3)	3/10 (30)	7/8 (87.5)	4/4 (100)	3/3 (100)	8/10 (80)

278 a. Number of positive animals/total analyzed (%).

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280

281

282 Figure1. Geographical distribution of antibody seroprevalence against CDV and CPV in
283 foxes (black circles) and wolves (white circles).

284