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Epizootiologic Investigations of Parvovirus Infections in Free-ranging Carnivores from Germany

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ABSTRACT: To assess if wild carnivores in Germany play a role in the epizootiology of canine parvovirus (CPV) infection, seroprevalences against CPV in free-ranging carnivores (n=1,496) from selected urban and rural areas were compared. Antibodies against CPV were found in sera from red foxes (*Vulpes vulpes*; 136 of 1,442; 9%), raccoon dogs (*Nyctereutes procyonides;* two of 33; 6%), stone martens (*Martes foina*; four of 13; 31%), and pine martens (*Martes martes*; one of two) using the hemagglutination-inhibition test and pig erythrocytes. Evidence of CPV infection was detected in all study areas. Antibody titers varied between 10 and 320. In red foxes, the number of reactors did not differ between most urban and rural areas. However, we found significantly more reactors in the most densely populated urban area (Berlin). None of 430 tissue samples (small intestine, spleen, mesenterial lymph nodes) from any species tested for the presence of CPV nucleic acid using polymerase chain reaction yielded an amplification product. Based on our results, we believe that contact between domestic dogs and free-ranging red foxes probably plays a subordinate role in the epizootiology of CPV in Germany.

Key words: Germany, *Martes foina, Martes martes,* parvovirus, *Nyctereutes procyonides,* polymerase chain reaction, serologic survey, *Vulpes vulpes.*

Two canine parvoviruses (CPV) infect dogs: minute virus of canines (MVC) and CPV-2. Both viruses belong to the genus *Parvovirus* within the family *Parvoviridae* (Appel et al., 1978). Canine parvovirus 2 probably arose by mutation from feline parvovirus (FPV) or a closely related virus. Canine parvovirus 2 is now recognized to contain two viruses classified as CPV-2a and CPV-2b. A red fox parvoviral sequence was intermediate between FPV and CPV (Truyen et al., 1998). In the order Carnivora, species from six families (Felidae, Canidae, Procyonidae, Mustelidae, Ursidae, and Viverridae) are suspected to be susceptible to CPV (Komolafe, 1986; Madic et al., 1993; Dunbar et al., 1998). Canine parvovirus infections occur with two major clinical syndromes. The first is marked by nonsuppurative myocarditis in pups <4 mo of age (McCandlish, 1981). The second is characterized by gastrointestinal signs and affects animals of all ages (Appel et al., 1978; Woods et al., 1980). Clinical signs include lethargy, depression, inappetence, vomiting, and diarrhea. Infected animals can excrete large amounts of virus in feces primarily 4–10 days after infection (Carman and Povey, 1985; Shen et al., 1986). The virus is transmitted by fecal-oral route (Appel et al., 1980). Many surveys for CPV infection in carnivores have been conducted (Steinel et al., 2001). However, the few reports on CPV infections in European native wildlife populations have been confined to red foxes (*Vulpes vulpes*) from Germany (Truyen et al., 1998), raccoon dogs (*Nyctereutes procyonides*) from Finland (Veijalainen, 1988), wolves (*Canis lupus*) from Italy (Martinello et al., 1997), and brown bears (*Ursus arctos*) from Italy and Croatia (Madic et al., 1993; Marsilio et al., 1997).

Our objective was to determine whether domestic dogs are a potential source of infection for free-ranging carnivores and vice versa. Therefore, we compared the prevalence of antibodies against CPV among free-ranging carnivores from selected urban and rural areas in Germany.

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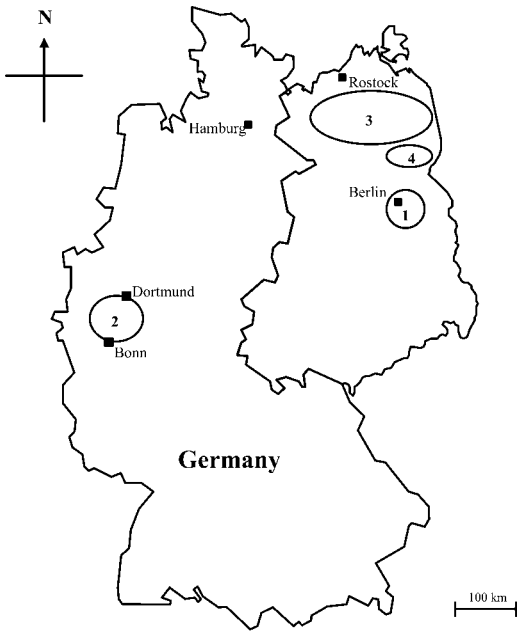

FIGURE 1. Distribution of study areas in Germany; urban areas in Berlin (1) and North Rhine-Westphalia (2); rural areas in Mecklenburg-Vorpommern (3) and Brandenburg (4).

In addition, we used polymerase chain reaction (PCR) to detect viral DNA.

Between January 1999 and June 2001, 1,496 serum samples and 430 tissue samples (small intestine, spleen, mesenterial lymph nodes) from red foxes (n=1,442), raccoon dogs (n=33), stone martens (*Martes foina*; n=13), badgers (*Meles meles*; n=6), and pine martens (*Martes martes*; n=2) were collected from selected areas in Germany. Five hundred and seventy-two sera originated from Berlin (52°30′N, 13°20′E), 265 from North Rhine-Westphalia (50°50′N to 51°40′N, 6°20′E to 7°30′E; both urban areas), 483 from Mecklenburg-Vorpommern (53°20′N to 53°60′N, 11°20′E to 13°50′E), and 176 from one area in Brandenburg (53°05′N to 53°20′N, 13°20′E to 14°10′E; both rural areas; Fig. 1). Sera and tissue samples were submitted by several state veterinary laboratories (Institut für Lebensmittel, Arzneimittel und Tierseuchen, Berlin, Germany; Landesveterinär- und Lebensmitteluntersuchungsamt Mecklenburg-Vorpommern, Rostock, Germany; Staatliches Veterinäruntersuchungsamt, Arnsberg; Staatliches Veterinär- und Lebensmittel- untersuchungsamt, Frankfurt/Oder, Germany) located in the respective study areas. Animals were shot or found dead by local hunters, and samples were taken at the veterinary laboratories within 48 hr after hunting. Sera were stored immediately at −20 C and tissue samples at −80 C. Serum and tissue came from the same animals. No clinical signs suggestive of parvoviral infection could be verified. We assumed a positive correlation between human population density and the density of domestic dogs (Frölich et al., 2000). Therefore, study areas with extremely high human population density were compared with areas with very low human population density (rural areas: Mecklenburg-Vorpommern, Germany, 41–49 persons/km^2; Brandenburg, Germany, 51 persons/km^2; urban areas: Berlin, Germany, 3,818 persons/km^2; cities in North Rhine-Westphalia, Germany, 1,242–2,376 persons/km^2). In addition, the densities of red foxes were estimated by different hunting indexes (hunting bag of the species/area [km^2]/hunting period; Bögel et al., 1974) for all study areas between 1999 and 2001. The hunting indexes of Mecklenburg-Vorpommern, Brandenburg, and North Rhine-Westphalia, Germany, varied between 1.09 and 1.69 and were not significantly different. In contrast, the hunting index in Berlin was significantly lower compared with all other areas (0.28).

For serologic examination, we performed a hemagglutination inhibition (HI) test as described by Carmichael et al. (1980). Briefly, sera were diluted 1:5 in barbital-borate albumin buffer (pH 6.2), inactivated at 56 C for 1 hr, and preadsorbed to pig erythrocytes. The prepared sera were subsequently incubated with four hemagglutinating units of CPV and were then incubated again for 1 hr at room temperature. As the last step, pig erythrocytes (0.5% in buffer solution) were added. Sera were considered to be positive at an HI titer of ≥10 (Truyen

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TABLE 1. Results of serologic survey for canine parvovirus in free-ranging carnivores from Germany.

Species	*n*	Antibody titer[a]					
		10	20	40	80	160	320
Red fox (*Vulpes vulpes*)	1,442	25	55	22	15	11	8
Raccoon dog (*Nyctereutes procyonides*)	33	2	—	—	—	—	—
Pine marten (*Martens martens*)	2	1	—	—	—	—	—
Stone marten (*Martens foina*)	13	—	2	2	—	—	—
Badger (*Meles meles*)	6	—	—	—	—	—	—

[a] Hemagglutination-inhibition test.

et al., 1998). The HI test used detects antibodies against FPV, MVC, and the various types of CPV-2, −2a, and −2b. These viruses are very closely related, and cross reactions can occur. However, there is no test available that will discriminate the specificity of parvovirus infection. Tissue samples (n=430) were examined for presence of viral DNA by PCR according to Steinel et al. (2000). For viral DNA preparation, a DNeasyTM Tissue Kit (Qiagen®, Hilden, Germany) was used. Viral DNA amplification was attempted by PCR using the primers M1 (GAAAACGGATGGGTGGAAAT) and M2 (AGTTGCCAATCTCCTGGATT). Potential differences between the antibody prevalences in different areas or the hunting seasons were evaluated using χ-square tests. Subsequently, calculated adjusted standardized residuals served to find the categories significantly deviating from the expected mean (Everitt, 1977). The comparison of titers was performed using the Kruskal-Wallis test and subsequent post hoc tests. The significance level was set to α=0.05. All statistical calculations were performed using SPSS 9.0 software (SPSS Inc., Chicago, Illinois, USA).

Of 1,496 free-ranging carnivores, 143 sera were positive for antibodies against CPV. The positive sera originated from red foxes, 136 of 1,442 (9%); raccoon dogs, two of 33 (6%); stone martens, four of 13 (31%); and pine martens, one of two. No positive sera were found in badgers (zero of six). Titers varied between 10 and 320 (Table 1).

Statistical analysis to compare antibody prevalences in different areas was performed for red foxes only. We found 13% of red foxes in Berlin to be seropositive (71 of 547), 8% in Mecklenburg-Vorpommern (38 of 474), and 6% in North Rhine-Westphalia (17 of 265), and Brandenburg (10 of 156). Significant differences between the regions (P=0.003, n=1,442) were due to a higher proportion of reactors in the extremely urban area of Berlin (adjusted standardized residual, SR=3.6) compared with the overall mean. Seroprevalences in Mecklenburg-Vorpommern (SR=−1.3), North Rhine-Westphalia (SR=−1.9), and in Brandenburg (SR=−1.4), did not differ from the expected mean. The proportion of reactors varied significantly between years. It was below the overall mean in the year 2000 (48 of 761, SR=−4.3) and above the mean in 1999 (54 of 416, SR=2.9) and 2001 (34 of 265, SR=2.1). Antibody titers were significantly higher (P<0.001, n=135) in 2001 (median=4.0) than in the other 2 yr (median=2.0 for both years, post hoc tests were significant for 2001 compared with both 1999 and 2000). The latter analysis was restricted to samples with positive titers only. Additionally, the prevalences for the species were compared separately for each study area. Restricting these comparisons on species with at least n=10 samples in the respective area, differences were found neither between foxes (71 of 547) and stone martens (four of 13) in Berlin (P=0.082) nor between foxes (10 of 156) and raccoon dogs (two of 20) in Brandenburg (P=0.630).

We found evidence of CPV exposure in red foxes, raccoon dogs, stone martens, and pine martens in different parts of Ger-

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many. This is in accordance with findings of Truyen et al. (1998), who found 13% seroprevalence in red foxes from Brandenburg between 1991 and 1995. The increase in seroprevalence in 2001 might indicate the onset of an epidemic. This is supported by significantly higher antibody titers in 2001 than in the previous 2 yr.

The higher than expected proportion of reactors in Berlin might indicate a possible transmission of CPV between domestic dogs and red foxes in this extremely urban area. It is unlikely that it reflects an association with red fox density, because the hunting index in Berlin was significantly lower than in all other areas. In the other study areas, the proportion of seropositive red foxes did not differ significantly between urban and rural regions, suggesting an independent infection cycle of CPV in red foxes and domestic dogs. This corresponds with the findings of Courtenay et al. (2001), who did not find evidence for transmission of CPV between domestic dogs and free-ranging, crab-eating foxes (*Cerdocyon thous*) in a study area in Brazil. Besides serologic methods, the contact-rate between crab-eating foxes and domestic dogs was documented by radiotracking. It was demonstrated that infected domestic dogs with either CPV or canine distemper virus (CDV) infection had contact with the foxes investigated. However, none of the crab-eating foxes in their study had antibodies against CPV or CDV. It was assumed that the potential risk of a CPV and CDV spill-over from domestic dogs to free-ranging carnivores might be lower than supposed.

The high proportion of seropositive stone martens (31%) might indicate a high infection rate within the stone marten populations studied.

Four hundred and thirty tissue samples were tested for specific CPV DNA. All tissue samples tested were negative. Negative PCR results may be explained by the fact that CPV infection is an acute disease. After infection, rapid antibody production is usually observed within 4 to 5 days. The detectable level of virus is already markedly reduced in tissue and fecal samples 7 to 9 days after infection (Carman and Povey, 1985). Thus, CPV DNA can only be identified in tissue within a short period after infection. Animals that recovered from CPV infection probably have complete and persistent immunity (Ackermann, 1981; Petermann and Chappuis, 1981). However, Truyen et al. (1998) were able to detect viral DNA in two of 51 red foxes with signs of acute gastroenteritis in Germany.

In conclusion, our results demonstrated evidence of exposure to CPV in red foxes, raccoon dogs, stone martens, and pine martens in different parts of Germany. For red foxes, the number of reactors did not differ between most urban and rural areas. Based on our results, we believe that contact between domestic dogs and free-ranging red foxes probably plays a subordinate role in the epizootiology of CPV in Germany.

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