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ABSTRACT: Canine distemper virus (CDV) has a wide host spectrum, and during the past years, distemper has been observed in species that were previously not considered to be susceptible. In this study, we investigated the prevalence of CDV-specific antibodies in red foxes (*Vulpes vulpes*) sampled between May and November 1997. About 9 to 13% of the Luxembourg red fox population is positive for antibodies against CDV. Thus a sizeable proportion of red foxes has been exposed to CDV in the wild. The significance of CDV in red foxes is discussed.

Key words: Canine distemper virus, red foxes, *Vulpes vulpes.*

Outbreaks of canine distemper continue to occur in domestic dogs in western Europe (Ek-Kommonen et al., 1997). Canine distemper is a highly contagious disease that affects not only dogs but also a wide range of carnivores, including stone martens (*Martes foina*), badgers (*Meles meles*), weasels (*Mustela* spp.), and red foxes (*Vulpes vulpes*) (Palmer et al., 1983; Hewicker et al., 1990; van Moll et al., 1995). Interspecies transmission frequently occurs and in recent years distemper has been observed in species that were previously not considered to be susceptible (Harder and Osterhaus, 1997). Infected dogs have been suspected as the primary source of infection, for instance, during a distemper epizootic in lions in East Africa (Roelke-Parker et al., 1996). Other authors wondered about the role of wildlife species as a potential source of canine distemper virus (CDV) for susceptible dog populations (Palmer et al., 1983; van Moll et al., 1995). There is at least one report of an outbreak in an incompletely vaccinated dog population following contact with a mustelid with canine distemper (Hewicker et al., 1990).

As a result of rabies vaccination in Luxembourg, the fox population has considerably increased with potential consequences for patterns of other infections. In the present study, we determined the prevalence of CDV antibodies in foxes in Luxembourg.

The total fox population in Luxembourg is estimated at about 10,000 animals with an average density of about four foxes/km^2 (Schon et al., 1992). In 1997, hunters were asked to submit the carcasses of foxes to the National Veterinary Institute of Luxembourg to monitor a rabies vaccination campaign. Blood samples were obtained by ventricular or cardiac puncture of mostly mature animals that were submitted between May and November 1997. Serum was separated from blood by centrifugation at 10,000×G for 30 min at room temperature and was stored at −20 C. Foxes originated from 10 of the 12 administrative regions of Luxembourg.

Anti-CDV antibodies were determined by virus neutralization test (NT). Serial two-fold dilutions of fox serum in Dulbecco's modified Eagle medium (DMEM; Gibco BRL, Paisley, UK) were incubated in 96-well flat bottom plates (Nunc, Denmark) for 1 hr at 37 C with 100 50%-tissue culture infective doses ($TCID_{50}$) of CDV (Bussel strain, ATCC, Rockville, Maryland, USA). Vero cells, in DMEM supplemented with penicillin/streptomycin and 2% fetal calf serum, were added to a final concentration of 10^4 cells per well. After incubation for 4 days in a humidified CO_2 incubator, the extent of neutralization was

TABLE 1. Detection of canine distemper virus antibodies detected by enzyme linked immunosorbent assay (ELISA; $n=6$) and serum neutralization assay (NT ($n=48$).

	ELISA positive (≥1:20)	ELISA negative (<1:20)
NT positive (≥1:20)	4	2
NT negative	4	34
NT (not tested)	0	17

determined microscopically as the reciprocal of the highest serum dilution that fully suppressed cytopathic effects. Anti-CDV IgG antibodies were measured in an indirect enzyme linked immunosorbent assay (ELISA), with protein A (Genzyme, Cambridge, Massachusetts, USA) as a conjugate. High-binding microtiter plates were coated with purified CDV treated with Triton X-100. One hundred μl of two-fold serial serum dilutions were added to each well and incubated for 1 hr at 37 C. Horseradish peroxidase-labeled protein A was then added at a 1:1,000 dilution and incubated for an additional hour. Plates were washed and developed by adding 100 μl tetramethylbenzidine as a substrate and optical density (OD_{450}) was measured at 450 nm. Results were considered positive when the OD value was more than three times the value of the negative control. A titer of <1:20 was considered negative in both tests. In both tests, CDV positive and negative dog sera were included as controls. All statistical tests were performed using StatXact 4.0 (Cytel Software Corporation, Cambridge, UK). The McNemar's test was used to test the equality of the binary response rates of the paired results of both tests.

Of 61 fox sera, only 44 were tested by NT due to the limited quality and quantity of some samples (Table 1). In the NT test, six sera (14%) with titers of 1:20–1:40 were considered CDV antibody positive. The 95% exact binomial confidence interval was 5.2–27.3%. Eight of 61 (18%) were positive in the ELISA (95% CI 8.2–32.7%). Four of 44 (9%) sera were positive in both tests. The agreement between the ELISA and the NT test was estimated to be moderate by exact Cohens Kappa test (kappa=0.49; 95% CI 0.14–0.84) according to the interpretation of Landis and Koch (1977). The difference between both tests was not deemed statistically significant by McNemar's test ($P=0.69$). While the NT test measures only antibodies against the hemagglutinin or the fusion protein, the ELISA also detects antibodies against other major proteins such as the nucleoprotein. Unspecific binding in ELISA or unspecific inhibition of virus proliferation could also account for discrepant results, although sera with cell toxicity were excluded from the NT test. When all 61 sera were considered, the proportion of sera positive by ELISA was 13% (95% CI 5.8–24.2%). Thus, CDV seropositivity in the surveyed fox population was estimated to be about 9–13%. Seropositive animals showed no regional clustering.

Interpretation of serologic surveys largely depends on the clinical course of the disease, which in the case of canine distemper may be different even in closely related species. The gray fox (*Urocyon cinereoargenteus*) is highly susceptible to CDV and in some natural mortality studies, more animals had died of distemper than of all other diseases combined (Davidson et al., 1992a, b). In red foxes, CDV caused severe systemic signs, including lung and brain lesions (Lopez-Peña et al., 1994) that were similar to those found in CDV infected dogs (Appel et al., 1981) and other species (Hewicker et al., 1990).

Other authors found little evidence of distemper in different populations of red foxes. Around Tokyo, no evidence of CDV infection or disease was found in red foxes during distemper epizootics in raccoon dogs (*Nyctereutes procyonides*; Machida et al., 1993). Similarly, in the US, red foxes showed only rare or no signs of distemper at necropsy, while most gray foxes were incubating the disease (Little et al., 1998; Davidson et al., 1992b). In Saarland, Ger-

many, which borders Luxembourg, van Moll et al. (1995) examined the brains of mustelids and red foxes found dead or killed near human habitat. Despite the high incidence in mustelids none of the red foxes had signs of acute or chronic encephalitis suggesting that either the infectivity or the susceptibility of infected animals to develop disease was low.

Our serologic survey conducted 7–8 yr later in neighboring Luxembourg, showed that red foxes have been exposed to CDV. This is similar to a serologic survey from Bavaria, Germany where 4.4% of CDV positivity was reported between 1991 and 1995 (Truyen et al., 1998). In Wisconsin, 11% of free-ranging red foxes were seropositive for CDV, while gray foxes from the same region were seronegative (Amundson and Yuill, 1981). In New York, 18% of red foxes had CDV-antibodies (Parker et al., 1961). In the above study by Davidson et al., (1992b) both red and gray foxes were seronegative.

Antibody prevalence of diseases such as distemper, which may have high mortality rates, may not reflect accurately the frequency of infection in the population. The case fatality rate was very low in wild red foxes (Little et al., 1998; Davidson et al., 1992b), although, by at least one account CDV caused severe pathology and death in captive red foxes (Lopez-Peña et al., 1994). If the prevalence of antibodies against CDV in (apparently healthy) trapped red foxes is 9–13%, the incidence of infection or disease may be higher depending on the proportion of animals that survived infection. Therefore, the role of red foxes in the epizootiology of CDV in both wildlife and domestic dogs in central Europe needs to be further explored.

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