

# SEROSURVEY FOR CANINE DISTEMPER VIRUS, CANINE ADENOVIRUS, *LEPTOSPIRA INTERROGANS*, AND *TOXOPLASMA GONDII* IN FREE-RANGING CANIDS IN SCANDINAVIA AND SVALBARD

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**ABSTRACT:** Prevalence of antibodies reactive to canine distemper virus (CDV), canine adenovirus type 1 (CAV-1), *Leptospira interrogans* serovars Canicola and Icterohaemorrhagiae, and *Toxoplasma gondii* were examined in free-ranging Scandinavian canids. Sampling included 275 red foxes (*Vulpes vulpes*) from mainland Norway, 60 arctic foxes (*Vulpes lagopus*) from the high-arctic islands of Svalbard, and 98 wolves (*Canis lupus*) from the joint Swedish-Norwegian population. Methods used included virus neutralization tests for CDV and CAV-1, a microscopic agglutination test for *L. interrogans*, and a direct agglutination test for *T. gondii*. High prevalence of antibody to CAV-1 was identified in red foxes (59.6%), wolves (67.7%), and arctic foxes (37.8%). The prevalence of antibody to CDV varied between 9.6% and 12.3% in the three species. Antibodies to *L. interrogans* serovar Icterohaemorrhagiae were found in 9.9% of the red foxes and 8.4% of the wolves sampled, whereas no antibody-positive arctic foxes were found. All animals were antibody-negative for *L. interrogans* serovar Canicola. Antibodies to *T. gondii* were found in 66.9, 51.7, and 18.6% of red foxes, arctic foxes and wolves, respectively. Significantly more adults than juveniles were antibody-positive for CDV in red foxes and arctic foxes, for CAV-1 in wolves, and for *T. gondii* in red foxes and wolves. There was a general tendency for adult female red foxes to have a higher prevalence of antibodies for CDV than adult males; this difference was statistically significant. The results indicate that CDV and CAV-1 are endemic in red foxes and wolves on the Scandinavian mainland and in arctic foxes on Svalbard. Although infection with *L. interrogans* serovar Icterohaemorrhagiae was relatively common in wild canids on mainland Norway, it was not found on Svalbard, where the maintenance host (*Rattus norvegicus*) is absent. All three species are commonly exposed to *T. gondii* through predation on infected intermediate hosts.

**Key words:** Adenovirus, arctic fox, distemper, *Leptospira*, red fox, serosurvey, *Toxoplasma*, wolf.

## INTRODUCTION

Three species of wild canids are present in Norway: the arctic fox (*Vulpes lagopus*), the wolf (*Canis lupus*), and the red fox (*Vulpes vulpes*). Although the arctic fox population of the high-arctic islands of Svalbard numbers several thousand individuals (Fuglei et al., 1998), both the arctic fox and the wolf are endangered and protected species in mainland Scandinavia (Kålås et al., 2006). On mainland Norway, the arctic fox population is estimated to be as low as 50 adult individuals (Eide et al.,

2008). Norwegian wolves belong to a joint Swedish-Norwegian population of about 200 animals (Wabakken et al., 2006). The red fox is widely distributed throughout mainland Norway and although there are no good estimates of the size of the red fox population, hunting statistics indicates a population size of 140,000–200,000 individuals in late summer (Eide and Sandal, unpubl.)

Until recently, there were no specific surveillance programs for infectious diseases in wild canids in Scandinavia and

reports of significant disease were largely restricted to sarcoptic mange in red foxes (Lindström et al., 1994) and rabies in arctic foxes on Svalbard (Mørk and Prestrud, 2004). Other viral diseases including canine distemper (CD) and hepatitis contagiosa canis (HCC, also called fox encephalitis) have been sporadically diagnosed in Scandinavian red foxes (Anonymous, 1965–2008; Borg, 1978). The bacterium *Leptospira interrogans* is known to cause infection in wild canids (Williams and Barker, 2001). Although leptospirosis was a common disease in domestic dogs in Norway 50 yr ago, it has not been reported in wild canids in Scandinavia (Strande, 1947). Leptospirosis in Norwegian dogs has been associated with both serovar Canicola with the dog as the maintenance host, and serovar Icterohaemorrhagiae with the brown or Norway rat (*Rattus norvegicus*) reservoir. The parasite *Toxoplasma gondii* has been associated with fatal infections in arctic foxes on Svalbard (Sørensen et al., 2005).

With the exception of serologic surveys for *T. gondii* in arctic foxes on Svalbard (Prestrud et al., 2007) and red foxes in Sweden (Jakubek et al., 2001) and a restricted area of Norway (Kapperud, 1978), such surveys for HCC, CD, *L. interrogans*, and *T. gondii* have not been performed on wild canid populations in Scandinavia. The purpose of the present work was to study the serologic presence of antibodies reactive to these agents in wild red foxes in Norway, arctic foxes on Svalbard, and in the Swedish-Norwegian wolf population.

**MATERIALS AND METHODS**

**Collection and preparation of serum samples**

Blood samples were obtained from 275 red foxes (1994–95, 2002–05) in Norway, 60 arctic foxes (1999–2001) from Svalbard, and 98 wolves (1998–2007) from the joint Swedish-Norwegian population (Table 1). Sex and geographic location were recorded, and age was determined by tooth eruption and degree

TABLE 1. Total number, age, and sex distribution of red foxes and wolves from Norway and Sweden, and arctic foxes from Svalbard, Norway, sampled for serum that was antibody-reactive to canine distemper virus, canine adenovirus type 1 virus, *Leptospira interrogans* serovars Canicola and Icterohaemorrhagiae, and *Toxoplasma gondii*, 1994–2007.

|                    | Red foxes<br>Norway | Arctic foxes<br>Svalbard | Wolves<br>Sweden and<br>Norway |
|--------------------|---------------------|--------------------------|--------------------------------|
| <b>Males</b>       |                     |                          |                                |
| Adults             | 101                 | 6                        | 29                             |
| Juveniles          | 59                  | 16                       | 17                             |
| <b>Females</b>     |                     |                          |                                |
| Adults             | 65                  | 8                        | 24                             |
| Juveniles          | 45                  | 21                       | 19                             |
| Unknown<br>sex/age | 5                   | 9                        | 9                              |
| <b>Total</b>       | <b>275</b>          | <b>60</b>                | <b>98</b>                      |

of tooth wear (juveniles <1 yr, adults >1 yr). The red fox blood samples were obtained from the thoracic cavity or heart of animals hunted primarily for parasitologic studies (Davidson et al., 2006). Red foxes were sampled from all regions of the country, with the majority from eastern Norway. The samples from arctic foxes on Svalbard (Spitsbergen, 76–81°N, 15–25°E) and the Swedish-Norwegian wolf population (54–69°N, 10–24°E), were collected from animals immobilized for biologic research purposes.

Blood samples were centrifuged and the serum stored at –20 C or –40 C. Serum samples were heat inactivated (56 C, 30 min) before testing. Not all sera could be tested for all agents because of poor quality or insufficient quantity of serum. The number of unsuitable serum samples was high for the virus neutralization tests against CD virus (CDV) and canine adenovirus type 1 (CAV-1) in red foxes.

**Virus neutralization tests for CDV and CAV-1**

For virus neutralization (VN) tests, CAV-1 and CDV (Bussel strain) were propagated in monolayer cultures of Madin-Darby canine kidney cells and Vero cells (African green monkey [*Cercoithacus aethiops*] kidney cell line; Norrby et al., 1970), respectively. Tissue cells were cultured in modified Eagle medium with Earle’s buffered saline solution (EMEM), supplemented with Tris buffer (16.4 mM), L-glutamin (4 mM), and gentamicin (100 µg/ml). The pH was adjusted to 7.6 and all incubations were at 37 C. All cell culture reagents were

from Cambrex (Walkersville, Maryland, USA). The cultures were monitored daily with an inverted microscope (Leitz, Wetzlar, Germany) for cytopathogenic effect (CPE), and when 75–100% CPE was observed after 5–7 days, the cultures were stored at  $-70^{\circ}\text{C}$ . The antigen was subsequently thawed and after centrifugation ( $1,400 \times G$ , 5 min), a 10-fold dilution series of the supernatant was added to a 96-well cell culture plate (Corning Costar®, Corning Life Sciences, Corning, New York, USA) containing tissue cells. The plate was incubated and examined daily for tissue culture infection and, if found positive, the tissue culture infectious dose resulting in 50% cell lysis ( $\text{TCID}_{50}$ ) was recorded. A dilution series of each serum sample together with virus (100 units of  $\text{TCID}_{50}$ ) was preincubated for 1 hr. Thereafter, 50  $\mu\text{l}$  of each dilution was added to duplicate microtiter wells of a cell-culture plate with freshly prepared tissue cells in 150  $\mu\text{l}$  EMEM with added heat-inactivated and  $\gamma$ -irradiated bovine fetal serum (100  $\mu\text{l}/\text{ml}$ ; Cambrex). Both positive and negative canine control sera were included in the assay. The plates were incubated up to 10 days. Sera resulting in CPE were retested in serial dilutions in twofold steps, and the highest serum dilution with a visible CPE was considered the sample titer. Titers  $\geq 1:4$  were considered positive (Appel and Robson, 1973).

#### Microscopic agglutination test for *Leptospira interrogans*

The standard method for conducting the microscopic agglutination test was used to quantify *Leptospira* antibodies from serum samples (Faine et al., 1999). The test antigens were *L. interrogans* serovars Canicola (strain Hond Utrecht IV) and Icterohaemorrhagiae (strain RGA) grown in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium (Difco™, Becton Dickinson, Sparks, Maryland, USA) at  $28^{\circ}\text{C}$  for 4–8 days (Ellinghausen and McCullough, 1965; Johnson and Harris, 1967). Serum samples were serially diluted in a sodium chloride solution (154 mM) in round-bottomed microtiter plates. Live antigen was added and the plates were incubated for 2 hr. Drops of all samples diluted 1:100 (final dilution with antigen added) were put on glass slides and screened for agglutination using a dark-field microscope (Leitz) at  $10\times$  magnification. Samples that reacted were retested in several twofold dilutions beginning at 1:50. The lowest dilution with at least 50% agglutination of spores was considered the sample titer. Samples with titers  $\geq 1:100$  were considered positive (OIE, 2004).

#### Direct agglutination test for *Toxoplasma gondii*

The serum samples were assayed for immunoglobulin G antibodies to *T. gondii* by a widely recognized direct agglutination test (Toxo-Screen, BioMérieux, Lyon, France). The sera were tested at dilutions 1:40 and 1:4,000; then retested in serial dilutions in threefold steps beginning at 1:60. Titers  $\geq 1:40$  were considered positive (Jakubek et al., 2001).

#### Statistical analysis

The chi-square test was used to evaluate differences in prevalence among sex and age categories.

## RESULTS

Antibodies reactive to CDV, CAV-1, and *T. gondii* were common in all three species examined (Table 2). The highest prevalences of antibodies to CAV-1 were in red foxes (59.6%) and wolves (67.7%), whereas the highest prevalences for antibodies to *T. gondii* were in red foxes (66.9%) and arctic foxes (51.7%). The prevalence of antibody to CDV varied between 9.6% and 12.3% in the three species. Prevalence of antibody to *L. interrogans* serovar Icterohaemorrhagiae was 9.9% and 8.4% in red foxes and wolves, respectively; no antibody-positive arctic foxes were found. All animals were negative for *L. interrogans* serovar Canicola antibodies.

Antibody titers against CAV-1 and *T. gondii* were very high in individual positive animals, whereas titers against CDV and *L. interrogans* serovar Icterohaemorrhagiae were generally low. Twenty-three red foxes, five arctic foxes, and 25 wolves had antibody titers  $> 1:128$  for CAV-1. For *T. gondii*, 20 red foxes and two arctic foxes had titers  $> 1:4,000$ .

For the two viruses, a generally higher percentage of adults than juveniles were found antibody-positive. The difference was statistically significant for CDV in red foxes ( $P < 0.05$ ) and arctic foxes ( $P < 0.01$ ), and for CAV-1 in wolves ( $P < 0.05$ ). There also was a tendency for adult female red foxes to be antibody positive more fre-

TABLE 2. Results of serum survey for antibodies reactive to canine distemper virus (virus neutralization test), canine adenovirus type 1 virus (virus neutralization test), *Leptospira interrogans* serovars Canicola and Icterohaemorrhagiae (microscopic agglutination test), and *Toxoplasma gondii* (direct agglutination test) in red foxes from Norway, wolves from Norway and Sweden, and arctic foxes from Svalbard, Norway.

|              | Canine distemper virus     |            | Canine adenovirus type 1   |            | <i>Leptospira interrogans</i> serovar |            |                            |            | <i>Toxoplasma gondii</i>   |            |
|--------------|----------------------------|------------|----------------------------|------------|---------------------------------------|------------|----------------------------|------------|----------------------------|------------|
|              | Positive/<br>No.<br>tested | % Positive | Positive/<br>No.<br>tested | % Positive | Canicola                              |            | Icterohaemorrhagiae        |            | Positive/<br>No.<br>tested | % Positive |
|              |                            |            |                            |            | Positive/<br>No.<br>tested            | % Positive | Positive/<br>No.<br>tested | % Positive |                            |            |
| Red foxes    | 7/57                       | 12.3       | 31/52                      | 59.6       | 0/202                                 | 0.0        | 20/202                     | 9.9        | 174/260                    | 66.9       |
| Arctic foxes | 5/43                       | 11.6       | 14/37                      | 37.8       | 0/60                                  | 0.0        | 0/60                       | 0.0        | 31/60                      | 51.7       |
| Wolves       | 9/94                       | 9.6        | 63/93                      | 67.7       | 0/95                                  | 0.0        | 8/95                       | 8.4        | 18/97                      | 18.6       |
| Total        | 21/194                     | 10.8       | 108/182                    | 59.3       | 0/357                                 | 0.0        | 28/357                     | 7.8        | 223/417                    | 53.5       |

quently than adult males; for CDV this difference was statistically significant ( $P < 0.05$ ). For *T. gondii*, more adults than juveniles were antibody positive, and in red foxes and wolves this difference was statistically significant ( $P < 0.05$ ).

**DISCUSSION**

This is the first report of antibody to CAV-1, CDV, and *L. interrogans* in free-ranging red foxes and wolves in Scandinavia and in arctic foxes in Svalbard, as well as antibody to *T. gondii* in the Scandinavian wolf population. Our data indicate that both CAV-1 and CDV are widespread in the red fox population in Norway and in the Swedish-Norwegian wolf population, as well as among arctic foxes on Svalbard. In light of this finding, it may seem strange that records of regular disease outbreaks in these populations are lacking, with only sporadic fatal cases having been diagnosed among red foxes (Anonymous, 1965–2008; Borg, 1978). This may reflect difficulty in detecting fatal cases in the wild, high resistance in the host, or a low virulence of the circulating viral strains. Moreover, wild canids should be regarded as a potential source of infection to unvaccinated dogs. The higher prevalence of antibody to CAV-1, compared to CDV, may be related to the greater environmental stability of CAV-1 (Williams and Barker, 2001). Our study also indicated

exposure of red foxes and wolves to *L. interrogans* serovar Icterohaemorrhagiae and all three species seemed to be commonly infected with *T. gondii*.

The highest prevalence of antibody to CDV was in red foxes (12.3%), which is in the upper range of that reported for this species in other European countries (3.5–13%; Truyen et al., 1998; Damien et al., 2002). A recent study from Spain showed an antibody prevalence of 17.1%, using enzyme-linked immunosorbent assay (Sobrino et al., 2008). The prevalence of antibody to CDV in arctic foxes on Svalbard was 11.6%, and little is known for this species from other studies. Ballard et al. (2001) tested 99 arctic foxes in Alaska, USA, by the VN test and found no antibody-positive animals. However, as the authors implied, most of the animals tested were young and may therefore not have been exposed to CDV at the time of sampling. The prevalence of antibody to CDV in wolves (9.6%) was within the range of comparative studies from North America (Choquette and Kuyt, 1974; Zarnke and Ballard, 1987). CDV is short-lived in the environment (Appel, 1987) and the relatively high antibody prevalence in foxes and wolves in our study presumably reflects continuous circulation of the virus within the fox and wolf populations or within other susceptible wild or domestic carnivores. In Germany, a higher antibody prevalence was identi-



fied in red foxes from urban compared to rural areas, indicating that free-ranging foxes may become infected following contact with domestic dogs (Frölich et al., 2000). On Svalbard, polar bears (*Ursus maritimus*) have been found to have antibody to CDV (Tryland et al., 2005).

The prevalence of antibody to CAV-1 found in our study was very high in red foxes (59.6%), wolves (67.7%), and arctic foxes (37.8%). Many of the animals also had high antibody titers, suggesting an endemic situation with the virus continuously circulating in these populations (Appel, 1987). Other studies in red foxes have shown much lower prevalences (3.5–23.2%; Amundson and Yuill, 1981; Truyen et al., 1998; Robinson et al., 2005). Comparative results for antibody to CAV-1 in arctic foxes are not available in the literature. In wolves, an antibody prevalence of 12.8–94.7% has been reported from North America (Choquette and Kuyt, 1974; Stephenson et al., 1982; Zarnke and Ballard, 1987).

We found 9.9% of the red foxes and 8.4% of the wolves had antibodies reactive to *L. interrogans* serovar Icterohaemorrhagiae. There were no animals with detectable antibody to serovar Canicola, and all arctic foxes tested from Svalbard were antibody-negative to both serovars. These results suggest the relatively common exposure of Scandinavian red foxes and wolves to serovar Icterohaemorrhagiae via rodents, either directly (by predation) or indirectly (by urine-contaminated soil, mud or surface waters), and little or no exposure to the dog-associated serovar Canicola. The absence of antibody-positive arctic foxes on Svalbard can be explained by the very restricted presence of rodents on these islands. Both serovars were relatively common causes of infection in dogs in Norway until 50 yr ago (Strande, 1947), after which they apparently vanished. However, a few cases of serovar Icterohaemorrhagiae infections in dogs have been diagnosed during the last decade, indicating that this infection may

be reemerging (Sunde et al., 2003). The prevalence of antibody to *Leptospira* in red foxes and wolves in our study can be compared to a small number of other studies in these species. In Germany, 0.4% of red foxes were found to have antibody to serovar Icterohaemorrhagiae (Müller and Winkler, 1994). In Minnesota, Khan et al. (1991) found 14.1% of wolves to have antibody to serovar Canicola, whereas 3.3% were positive for antibody to serovar Copenhageni, which cross-reacts serologically with serovar Icterohaemorrhagiae. In Alaska, Zarnke and Ballard (1987) reported 1.2% of wolves positive for antibody to serovar Icterohaemorrhagiae. The impact of leptospirosis in free-ranging canids is largely unknown (Williams and Barker, 2001), and the generally low antibody titers in antibody-positive animals in this study may indicate a low clinical significance of infection.

The prevalence of antibody to *T. gondii* identified in this study was high for both red foxes (66.9%) and arctic foxes (51.7%), and lower in wolves (18.6%). High antibody prevalence in free-ranging carnivores is to be expected, considering they are predators or scavengers of a number of potentially infected intermediate hosts (rodents, cervids, and birds) containing tissue cysts of the parasite. Vikøren et al. (2004) found 39.9, 12.6, and 7.7% of Norwegian roe deer (*Capreolus capreolus*), moose (*Alces alces*), and red deer (*Cervus elaphus*), respectively, to have antibody to *T. gondii*. In arctic foxes on Svalbard, preying and scavenging on birds is considered an important source of infection (Prestrud et al., 2007). The higher antibody prevalence in foxes compared to wolves might be linked to the more opportunistic feeding behavior of the fox. The antibody prevalence in red foxes was higher than previously identified in Scandinavian studies (31.0 and 38.8% by Kapperud [1978] and Jakubek et al. [2001], respectively). In other parts of Europe, antibody prevalences of 47.1–98.4% have been reported (Buxton et al.,

1997; Wolfe et al., 2001). The antibody prevalence in arctic foxes on Svalbard was a little higher than that found in another recent study (43%; Prestrud et al., 2007), and the prevalence in wolves was higher than previously identified in Alaska (8.8%; Zarnke et al., 2000). Little is known of the clinical significance of *T. gondii* infection in free-ranging canids, but single fatal cases have been reported in red foxes both in Europe (Møller, 1952) and in North America (Dubey et al., 1990), as well as in the arctic fox population on Svalbard (Sørensen et al., 2005).

In conclusion, results of this serologic study indicate that CDV and CAV-1 are present in the wild red fox and wolf populations in Scandinavia, and in the arctic fox population of Svalbard. All three species seem to be frequently infected with *T. gondii* through predation on infected intermediate hosts. Exposure to *L. interrogans* serovar Icterohaemorrhagiae by rodent predation also seems to occur in red foxes and wolves. In the future, studies should be done to identify and characterize the causative microorganisms involved in infection, creating a better understanding of the natural history and epidemiology of these infections in free-ranging canids.

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