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Survey for *Encephalitozoon cuniculi* in Arctic Foxes (*Alopex lagopus*) in Greenland

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ABSTRACT: Wild arctic foxes (*Alopex lagopus*) from Greenland were tested for antibodies to *Encephalitozoon cuniculi* with an enzyme-linked immunosorbent assay and a carbon immunoassay. Of 230 tested foxes none was seropositive. This finding contrasts with observations from other arctic areas and absence of rodents in the diet of these arctic foxes is the most likely explanation for absence of *E. cuniculi*.

Key words: *Alopex lagopus*, arctic fox, *Encephalitozoon cuniculi*, Greenland, microsporidia, parasitic zoonosis, survey.

Encephalitozoon cuniculi is a microsporidian parasite of vertebrates which has been identified in a wide range of mammals (Canning and Lom, 1986). Disease occurs in animals having depressed immunity, either natural in the course of development of the immune system, or due to a contemporary infection. Encephalitozoonosis is seen in young rabbits and carnivores after transplacental infection (Flatt and Jackson, 1970; Mohn et al., 1982b). As a zoonotic pathogen, *E. cuniculi* is one of several microsporidia species that have been identified in humans with acquired immunodeficiency syndrome (AIDS; Weber et al., 1994).

Subclinical infection usually occurs after oral transmission in arctic foxes (*Alopex lagopus*) (Mohn et al., 1982b). Vertical transmission occurs when *E. cuniculi* infects and passes the placenta. As a result the pups become infected and develop encephalitozoonosis when they are 1–3 mo old (Mohn et al., 1974). Neurological signs dominate the clinical picture with limping, head tilt and circling movements. Pups suffering convulsions frequently lie on one side. Blindness can occur. If the disease is acute, clinical signs are severe, and pups die suddenly. Chronic cases survive, but pups develop poorly. The foxes lose appetite and are ataxic. Affected animals are thirsty due to kidney damage (Nordstoga, 1972). Hypersensitivity reaction results in polyarteritis nodosa and is observed in blood vessels of the brain and heart at postmortem examination (Nordstoga and Westbye, 1976).

Encephalitozoonosis has been a threat to farmed arctic foxes in Scandinavia since the late 1960s and most cases have been attributed to food contaminated by infected rodent carcasses (Nordstoga, 1972; Nordstoga et al., 1974). Rodents are latently infected and shed parasites in the urine. The most common mode of transmission in rodents is by ingestion of infected or contaminated feed, including prey, or by infected urine (Canning and Lom, 1986). Molecular studies have identified three strains of *E. cuniculi* (Didier et al., 1995). One strain was isolated from rabbits and humans, another from arctic foxes and mice, and a third from dogs and humans (Hollister et al., 1996; Mathis et al., 1996, 1997).

In the wild, the arctic fox has a circumpolar distribution. The southern border of its distribution is probably limited by the competing red fox (*Vulpes vulpes*). The arctic fox is an opportunistic predator and scavenger, and little is known about how diet and population density correlates with prevalence of *E. cuniculi* infection. In Iceland, Hersteinsson et al. (1993) diagnosed encephalitozoonosis in caged pups taken from the wild. Furthermore, they investigated 372 wild arctic foxes for antibodies to *E. cuniculi*; 41 of these were seropositive. Seroprevalence varied from 2–27% in the northeastern part of Iceland. Mice (*Apodemus sylvaticus* and *Mus musculus*) and mink (*Mustela vison*) from the same

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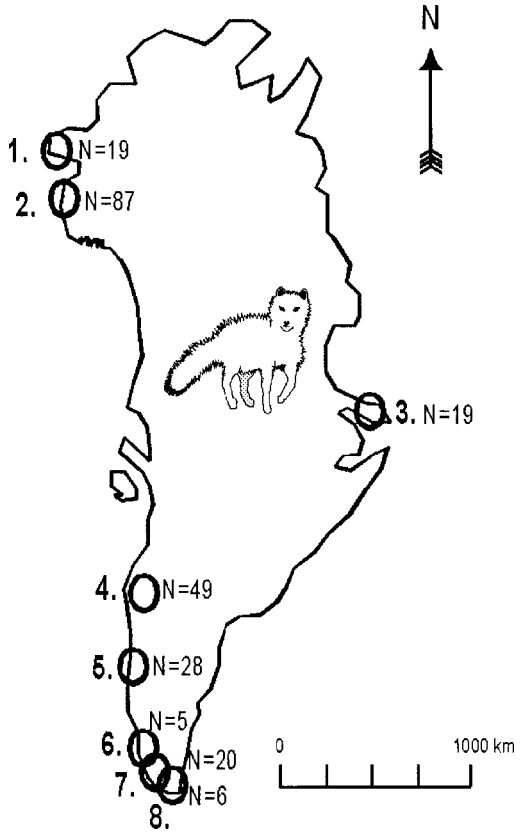


FIGURE 1. Geographic origin and number of arctic foxes (*Alopex lagopus*) from Greenland tested for *E. cuniculi* antibodies. Sampling areas: Siorapaluk (1), Thule Air Base (2), Scoresbysund (3), Kangerlussuaq Air Base (4), Nuuk district (5), Narsaq district (6), Qaqortoq district (7) and Nanortalik district (8).

areas where foxes were hunted were also seropositive. To our knowledge, rodents living in Greenland have not been tested for *E. cuniculi* infection. However, rodents are rarely consumed by arctic fox in Greenland. The aim of this study was to investigate the seroprevalence of *E. cuniculi* infection in arctic foxes in Greenland.

Blood samples were obtained from 230 arctic foxes from Greenland (59°46′–88°40′N; 12,10°–74,20°W, covering 2,175,600 square km; Fig. 1). During 1992–93, local Inuit hunters trapped 209 foxes for subsistence purposes and another 21 were shot through a governmental initiative to control the fox population. The foxes came from eight different geographical regions (Fig 1), and 121 vixens and 109 males were caught. There were 128 blue and 92 white-color-phase foxes aged 0–11 yr (mean 2.9 yr; Kapel, 1995).

Foxes were frozen and transported to Denmark prior to sampling. Blood was drawn from thawed carcasses. After centrifugation at 2,000×G for 20 min, serum was collected. The samples were partly hemolyzed.

All sera were tested for antibodies against *E. cuniculi* with an indirect enzyme linked immunosorbent assay (ELISA) described in detail elsewhere (Åkerstedt, 2002). Soluble antigen from *E. cuniculi* isolated from a blue fox (Mathis et al., 1996) was coated to microtiter plates. The serum samples were diluted 1:100 in phosphate buffered saline (PBS: 170 mM, pH 7.4), with tween 20 (50 μg/ml) and skimmed milk (30 mg/ml), and were added to two wells each. The conjugate was horseradish peroxidase (HRP) conjugated goat anti-dog IgG (whole molecule; Cappel 55334; 1:5,000), and the substrate was 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS™, Sigma-Aldrich, St. Louis, Missouri, USA). Results were expressed with the mean sample to positive ratio (S:P-ratio=[OD_{405} of sample−OD_{405} of negative control]/[OD_{405} of positive control−OD_{405} of negative control]) . Control sera were pooled from three farmed foxes with and five farmed foxes without clinical disease (Åkerstedt, 2002). Cut-off values were selected by a two-graph receiver operating characteristic (TG-ROC) plot (Greiner et al., 1995) of 205 tested farmed blue foxes. Sample to positive ratio >0.151 was considered positive, values <0.116 were negative; whereas values between these limits were considered intermediate. A sample that had two S:P values on both sides of a cutoff value was not classified (uninterpretable).

Sera with positive, intermediate, or uninterpretable results in the ELISA and a number of randomly chosen negative samples were tested with the carbon immunoassay (CIA; Waller, 1977). The test was

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performed according to the manufacturer's instructions (Testman, Uppsala, Sweden). Each sample was diluted 1:50 with PBS, and the ELISA control sera were used. The diluted serum was mixed with 3×10^5 *E. cuniculi* spores and incubated at room temperature for 2 min. The suspension was mixed with India ink on a glass slide and covered by a cover glass. The result was read immediately in a light microscope at 1,000× magnification with oil. Carbon particles in the India ink were bound to IgG of the sample thus visualizing specific binding of antibodies to *E. cuniculi* spores. With positive sera, the *E. cuniculi* spores were stained grayish-black against the background of carbon particles, and with negative sera, the parasite appeared white on the dark background. Sera that agglutinated spontaneously in the test were diluted 1:100 and retested.

The ELISA S:P values from the arctic foxes from Greenland were compared by the Mann Whitney test (Altman, 1993) with the results of 104 seronegative farmed blue foxes tested previously (Åkerstedt, 2002).

Results of the 230 tested sera from arctic foxes are shown in Figure 2. The median ELISA S:P value was −0.060 and 95% of the observations were between −0.094 and 0.046. Of the foxes, 225 were negative and had S:P values in the range from −0.097–0.032. Results of five foxes could not be classified. These and nine of the ELISA negative foxes, randomly chosen, were negative in the CIA test. The ELISA S:P values were significantly ($P<0.001$) lower for the arctic foxes than for seronegative farmed blue foxes from Norway.

Results of the present study indicate that *E. cuniculi* infection is not present in arctic foxes in Greenland. Unlike in Iceland, where mice and arctic foxes live in the same areas (Hersteinsson et al., 1993), only one species of rodent, the collared lemming (*Dicrostonyx torquatus groenlandicus*), and one species of lagomorph, the arctic hare (*Lepus arcticus*) are known to occur in Greenland. Arctic hares are of minor importance in the diet of the arctic foxes (Birks and Penford, 1990; Kapel, 1995). Transmission of *E. cuniculi* could potentially occur between lemmings and arctic foxes. Lemmings are only found in the tundra areas of the Northeast Greenland National Park, where they are the main prey of arctic foxes. In the present study, remains of lemmings were not found in the gastrointestinal contents of foxes derived from Scoresbysund (70°28′N, 21°46′W), Central East Greenland ($n=16$; Kapel, 1995). Thus, the absence of infected rodents in the diet of the arctic foxes examined in the present study is the most likely explanation for the absence of *E. cuniculi*.

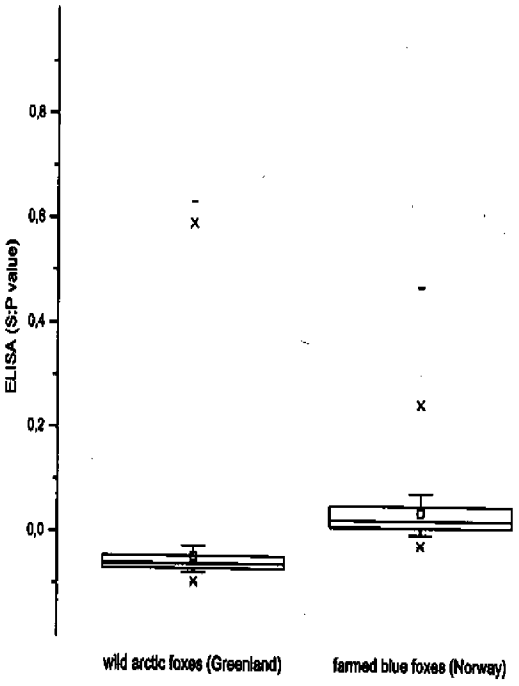


FIGURE 2. Box-plot of *E. cuniculi* ELISA results of 230 arctic foxes compared to 104 negative blue foxes verified with IFAT. The horizontal lines in the box denote the 25th, 50th, and 75th percentile values. The error bars denote the 5th and 95th percentile values. The symbols below the 5th percentile error bar denote the 0th (−) and 1st (×) percentile values. The two symbols above the 95th percentile error bar denote the 99th (×) and 100th (−) percentiles. The square symbol in the box denotes the mean ELISA S:P value of the tested animals.

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Humoral immune response to *E. cuniculi* is strong and antibody titers in naturally infected animals with clinical signs of encephalitozoonosis are usually high (Mohn, 1982). Animals with subclinical infection usually also have high titers, but some individual animals may have low titers (Mohn et al., 1982a). The cutoff value of a serologic test has to be established so that known infected and antibody producing animals are positive, and non-infected are negative in the test. For epidemiologic studies, where the prevalence is investigated serologically, false positive results may be crucial. This is especially the case when the true prevalence is low. Indirect immunofluorescence (IIF), CIA, and ELISA have similar specificities and sensitivities for *E. cuniculi* (Boot et al., 2000; Åkerstedt, 2002), and both IIF and CIA have proven successful in monitoring and establishing rabbit colonies free of encephalitozoonosis (Cox et al., 1977; Bywater and Kellett, 1978), where it was important to avoid false negative results. Since the introduction of CIA for farmed blue foxes (Mohn and Ødegaard, 1977), it has been the most used diagnostic method for *E. cuniculi* in this species. The CIA is simple to perform, requires no special equipment and can be quickly executed. However, the test is nonspecific, and the mechanism by which carbon particles attach to IgG is unknown. The CIA occasionally induces spontaneous agglutination of sera at lower dilutions and may therefore falsely be interpreted as positive. From the experience in our laboratory, dilution of 1:25 of hemolyzed sera may cause agglutination at a rather high rate. Thus, for routine testing and for examination of the samples in the present study, the dilution 1:50 was used.

The reason for lower ELISA values in the present study than for farmed blue foxes without *E. cuniculi* infection is not clear. The consequence of the suboptimal handling of the blood samples cannot be excluded, but on the other hand Hersteinsson et al. (1993) treated the blood in a similar way in their study and found seropositive animals. Antigenic similarities of the spore wall of microsporidia make strong antibody cross-reactions possible between different species (Niederkorn et al., 1980; Weiss et al., 1992). Microsporidia are parasites of virtually all vertebrates and feed of animal origin may contain relatively large amounts of microsporidian antigen (Canning and Lom, 1986). Thus, diet could influence serologic cross-reactions. Animals could also be exposed to microsporidia through helminths and insects, and the composition of gastrointestinal helminths shows distinct differences geographically (Kapel and Nansen, 1996). In general, the infectious pressure through microorganisms and parasites is low in the Arctic region, which as a result gives a weak stimulation of the immune system.

Arctic foxes are very susceptible to encephalitozoonosis, while farmed red foxes do not acquire disease (Nordstoga et al., 1974). The reason for this could be that in most places *E. cuniculi* has not been a threat to arctic foxes during their evolution. In this way, the host and the parasite have not adapted to each other, and the fox has not developed appropriate defense mechanisms. Coexistence with mice as a reservoir for *E. cuniculi* could be of a later date, and where mice infect arctic foxes, the parasite may cause severe disease.

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