RESEARCH ARTICLE

Why are Svalbard Arctic foxes *Brucella* spp. seronegative?

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Abstract

Arctic foxes (*Vulpes lagopus*) are susceptible to smooth *Brucella* (s-*Brucella*) infection and may be exposed to such bacteria through the consumption of infected marine mammals, as implied by the finding of s-*Brucella* antibodies in polar bears (*Ursus maritimus*). Arctic foxes in Svalbard have not previously been investigated for s-*Brucella* antibodies, but such antibodies have been detected in Arctic foxes in Iceland, Alaska (USA) and Russia. We investigated blood from Svalbard Arctic foxes for s-*Brucella* antibodies using an indirect enzyme-linked immunosorbent assay (iELISA). The animals (0–13 years old) were either caught by fur trappers (1995–2003, n = 403) or found dead (1995 and 2003, n = 3). No seropositive animals were detected. Morbidity and mortality due to the infection cannot be ruled out. However, no known, large disease outbreaks of unknown aetiology have been reported. Furthermore, it is unlikely that the Svalbard Arctic fox is resistant to infection as Arctic foxes from other populations are susceptible, and there is circumpolar connectivity between populations. The discrepancy between the findings in Iceland and Svalbard is surprising as both populations are on islands with no known local sources of exposure to s-*Brucella* other than marine mammals. However, our negative findings suggest that marine mammals may not be a major source of infection for this species. Comparative investigations are needed in order to draw conclusions regarding the epizootiology of s-*Brucella* in Arctic foxes in Svalbard and Iceland.

Keywords

Infection; serology; smooth *Brucella*; s-*Brucella*; *Vulpes lagopus*; epizootiology

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Abbreviations

s-*Brucella*: smooth *Brucella*
iELISA: indirect enzyme-linked immunosorbent assay
LPS: lipopolysaccharides
r-*Brucella*: rough *Brucella*
SD: standard deviation
%P: percent positivity

Introduction

The High-Arctic archipelago of Svalbard (78–81°N, 10–30°E) is located midway between the Norwegian mainland and the North Pole. The Arctic fox (*Vulpes lagopus*) is the only terrestrial mammalian predator in Svalbard (Fuglei et al. 2002). It is almost omnipresent, and the population is relatively stable, with no long-term population trends but with significant year-to-year variations (Nater et al. 2021). Arctic foxes in Svalbard belong to the coastal ecotype, being generalists in an ecosystem lacking cyclically fluctuating small mammal populations, feeding from both the marine and the terrestrial food webs (Fuglei & Ims 2008). Arctic foxes hunt ringed seal (*Pusa hispida*) pups and follow polar bears (*Ursus maritimus*) on the sea ice, scavenging remains of seals killed by bears (Frafjord 1993). Analysis of the stomach content of 898 Svalbard Arctic foxes revealed the remains of seal in 2.5% of the stomachs (Prestrud 1992), and stable isotope analysis revealed that marine resources, such as sea birds or marine mammals, are used by Arctic foxes in Svalbard (Ehrich et al. 2015). Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and rock ptarmigan (*Lagopus muta hyperborea*) also constitute large parts of the Arctic fox diet all year round (Prestrud 1992; Fuglei et al. 2002; Eide et al. 2005; Eide et al. 2012). In spring and summer, additional food items are various birds and eggs (Frafjord 1993; Eide et al. 2005).

Brucella species occur in both smooth (s-*Brucella*) and rough (r-*Brucella*) forms, depending on the presence or absence of an O-polysaccharide on the cell surface, which influences virulence (Rittig et al. 2003). S-*Brucella* species include amongst others *Brucella suis, Brucella ceti* and *Brucella pinnipedialis.* S-*Brucella* may infect and cause disease in a range of marine mammal species and populations (Nymo et al. 2011). Antibodies against s-*Brucella*

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Table 1 Isolation of *Brucella* spp. and the detection of antibodies against smooth *Brucella* spp. in seals, whales and polar bears in waters surrounding Svalbard and other areas.

Species[a]	Antibodies Svalbard[b]	Isolation other areas[b, c]	Antibodies other areas
Atlantic walrus (*Odobenus rosmarus rosmarus*) P	Scotter et al. 2018	No	Nielsen et al. 2001[d]
Bearded seal (*Erignathus barbatus*) P	Foster et al. 2018	Foster et al. 2018	Foster et al. 2018
Harbour seal (*Phoca vitulina*) P	No	Foster et al. 2002[d]	Nymo et al. 2018[d]
Harp seal (*Pagophilus groenlandicus*) S	Tryland et al. 1999[d]	Forbes et al. 2000[d]	Nielsen et al. 2001[d]
Hooded seal (*Cystophora cristata*) S	Tryland et al. 1999[d]	Foster et al. 1996	Nielsen et al. 2001
Ringed seal (*Phoca hispida*) P	Tryland et al. 1999	Forbes et al. 2000[d]	Nymo et al. 2018[d]
Atlantic white-sided dolphin (*Lagenorhynchus acutus*) S	No	Foster et al. 2002[d]	No
Beluga (*Delphinapterus leucas*) P	No	Whatmore et al. 2017	Nielsen et al. 2001[d]
Bowhead whale (*Balaena mysticetus*) P	No	No	No
Fin whale (*Balaenoptera physalus*) S	No	No	Tryland et al. 1999
Killer whale (*Orcinus orca*) S	No	Raverty et al. 2002	Jepson et al. 1997[d]
Minke whale (*Balaenoptera acutorostrata*) S	No	Tryland et al. 1999[d]	Tryland et al. 1999[d]
Narwhale (*Monodon monoceros*) P	No	No	Nielsen et al. 2001
White-beaked dolphin (*Lagenorhynchus albirostris*) S	No	Foster et al. 2002	No
Polar bear (*Ursus maritimus*) P	Tryland et al. 2001	No	Atwood et al. 2017[d]

[a]Permanently (P) or sporadically (S) present in waters surrounding Svalbard. [b]A citation indicates a positive finding. [c]The only host species from which *Brucella* spp. has been isolated in the Svalbard area is the hooded seal (*Cystophora cristata*; Nymo, Tryland et al. 2013; Tryland et al. 2005), and the column indicating isolation from Svalbard has been omitted from the table to save space. [d]Other references exist in addition to this reference.

have been detected in numerous marine mammal species inhabiting the waters surrounding Svalbard (Table 1), signifying that the bacteria are circulating in these species. The seal-specific strain *B. pinnipedialis* has been isolated at a high prevalence from hooded seals (*Cystophora cristata*) caught in the so-called West Ice area, between Svalbard and Greenland (Tryland et al. 2005). S-*Brucella* antibodies have been detected in polar bears in Svalbard, with a seroprevalence of 4% in animals captured on land and 16% in polar bears captured on the sea ice east of Svalbard, suggesting ingestion of marine mammals as a source of infection (Tryland et al. 2001). Similarly, a study of polar bears from the Beaufort Sea population showed that animals that remained on the sea ice during summer had 2.5 times higher odds of being s-*Brucella* seropositive as compared to polar bears in more land-based habitats (Atwood et al. 2017).

Arctic foxes are susceptible to infection with s-*Brucella*. *Brucella suis* biovar 4, which can cause brucellosis in reindeer/caribou (*Rangifer*; Josefsen et al. 2018), has been isolated from Arctic foxes in Russia (Zheludkov & Tsirelson 2010) and Alaska (Morton 1986). Arctic foxes harboured the bacterium after being fed reindeer meat with *B. suis* biovar 4 (Pinigin et al. 1970), and seropositive (50%, n = 4) wild Arctic foxes have been detected in areas with *B. suis* biovar 4 in *Rangifer* spp. (Morton 1986, 1989). In Iceland, s-*Brucella* has not been detected in any terrestrial species (European Centre for Disease Prevention and Control 2019), yet s-*Brucella* antibodies were detected in Icelandic Arctic foxes in a study by Czirják et al. (2016). The coastal foxes had a higher seroprevalence (63%) than the inland foxes (25%), and the authors suggested marine mammal meat as the source of infection.

Seropositive wild red foxes (*Vulpes vulpes*) have been described, and the source of infection was thought to be *B. suis* biovar 4 from infected *Rangifer* (Neiland 1975; Morton 1986, 1989; Zheludkov & Tsirelson 2010). *Brucella suis* biovar 4 has also been isolated from wild red foxes. Moreover, red foxes that were orally challenged with *B. suis* biovar 4 harboured antibodies for several months, and *B. suis* biovar 4 was isolated from tissues (Morton 1986). *Brucella suis* biovar 2 has also been isolated from red foxes, with the European brown hare (*Lepus europaeus*) the likely source of infection (Hofer et al. 2010). Other wild carnivore species are also susceptible to infection with s-*Brucella* and may seroconvert following exposure (Kosoy & Goodrich 2018).

As we know that both polar bears and Arctic foxes have a high degree of contact with, and consumption of, marine mammals (Prestrud 1992; Iversen et al. 2013), both species should be commonly exposed to s-*Brucella*. Moreover, a high seroprevalence has been found in Arctic foxes in Iceland (Czirják et al. 2016), and marine mammals were suggested as the source of infection. The aim of our study was to investigate whether Svalbard Arctic foxes are exposed to s-*Brucella* spp., and which

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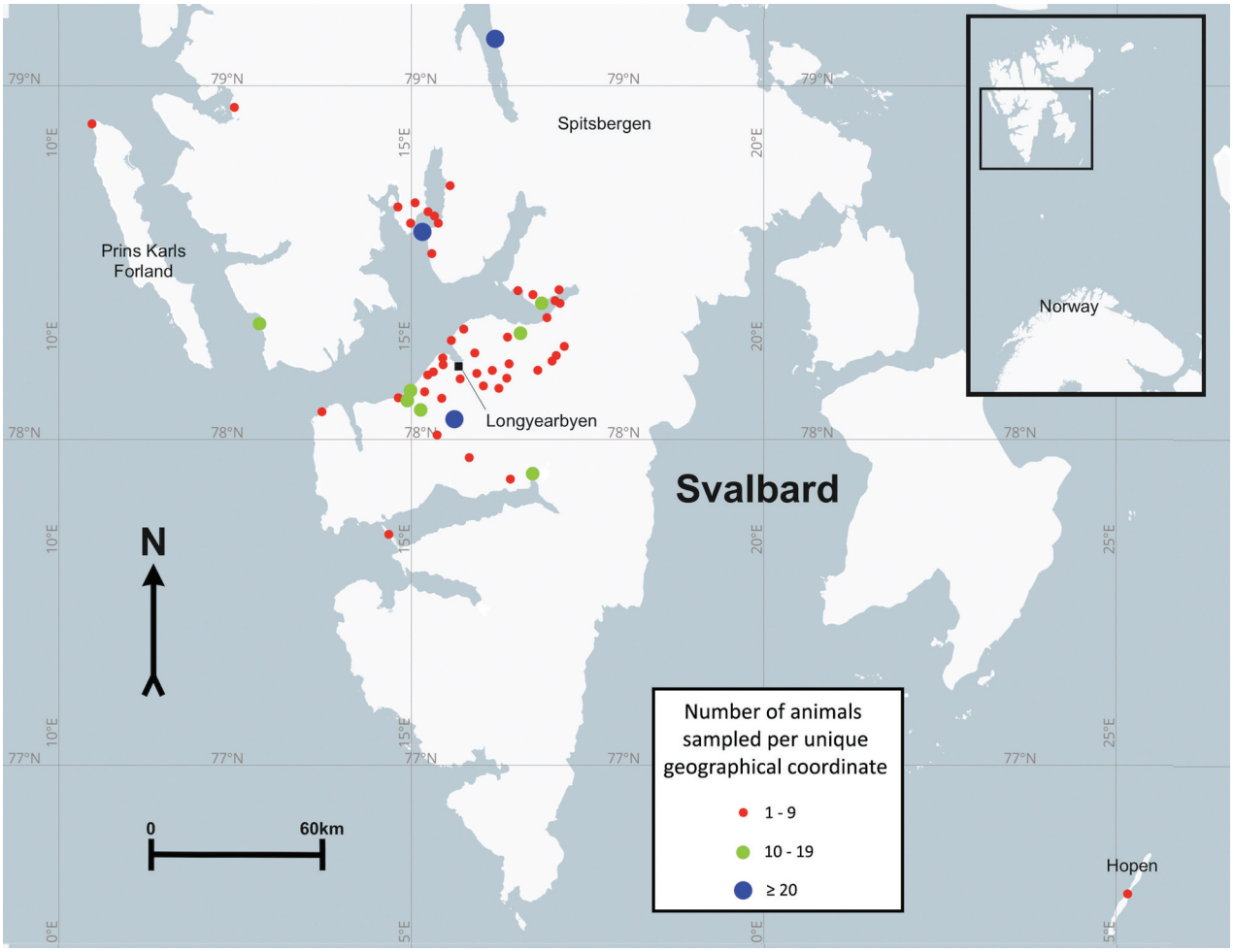

Fig. 1 Geographic distribution of Svalbard Arctic foxes investigated for smooth *Brucella* spp. antibodies. (The figure was made using ©Mapbox, ©OpenStreetMap; www.mapbox.com; Washington, DC, and San Francisco, CA).

geographical and physical traits affected their likelihood of seropositivity.

Materials and methods

Arctic foxes (n = 403) were caught in baited traps by fur trappers during winter (1996–2003). Three Arctic foxes found dead in 1995 and 2003 were also investigated. The Arctic foxes were frozen at −80 °C for minimum seven days to neutralize the eggs of the parasite *Echinococcus multilocularis*. The carcasses were then stored at −20 °C until necropsy. Sex (n = 405) and age (Grue & Jensen 1976) were determined and noted (n = 316). Blood was obtained (n = 406) from the heart or the thoracic or abdominal cavity during necropsy and centrifuged at 3500 rpm for 15 minutes, and the serum was stored at −20 °C until analysis.

Serum samples (n = 406) were analysed for s-*Brucella* antibodies with iELISA, a protein A/G iELISA (Nymo, Godfroid et al. 2013). A serum sample from a *B. pinnipedialis* bacteriology positive hooded seal (Tryland et al. 2005) was included on each plate as a positive control. The mean optical density of duplicate wells was expressed as a percentage of the reactivity of the positive control: ([optical density of the sample/optical density of the positive control] × 100) = %P. The cut-off was based on the mean value of the %P for hooded seal samples that were classified seronegative in the complement fixation test, the slow agglutination of Wright with ethylenediamine tetra-acetic acid and the Rose Bengal test plus 2.58 SDs. This provided a cut-off of 73.6 %P.

Descriptive statistics (i.e., range, mean and SD) were performed using the statistical analysis software JMP 14 (SAS Institute).

Results

The captured foxes (n = 403, 186 females, 216 males and one unknown) and the three foxes found dead (two males and one female) were obtained from various locations in Svalbard (Fig. 1); geographic origin was unknown for nine animals. Both inland (n = 45) and coastal (n = 307) resource areas were represented in all trap seasons (1996–97: nine coast/one inland, 1997–98: 42 coast/four inland, 1998–99: 94 coast/11 inland, 1999–2000: 53 coast/11 inland, 2000–01: 21 coast/one inland, 2001–02: 87 coast/16 inland, 2002–03: one coast/one inland).

The captured foxes for which age could be determined (n = 313) were one year of age (n = 153), two (n = 84), three (n = 33), four (n = 10), five (n = 9), six (n = 5), seven (n = 6), eight (n = 8), 10 (n = 2), 11 (n = 2) and 13 (n = 1) years old. Carcass weights (n = 279; 1500–5100 g) and fat indexes (visible amount of subcutaneous and abdominal fat [Prestrud & Nilssen 1992]) were registered. The captured foxes were categorized as having fat index 0 (no fat, n = 16), 1 (low, n = 80), 2 (moderate, n = 130), 3 (considerable, n = 120), 4 (extensive, n = 54) and not registered (n = 3).

Of the three animals found dead, a male pup and a juvenile male were emaciated, so the cause of death was presumed to be starvation. The cause of death could not be determined for the third fox, an adult female in good condition.

All Arctic foxes were classified as seronegative. The iELISA results ranged 0.3–5.3 %P (mean 1.7 %P, SD = 0.9 %P).

Discussion

All Arctic foxes investigated in this study were seronegative. This finding was surprising as s-*Brucella* antibodies have been detected in marine mammal species in Svalbard waters. Many of these marine mammals were found to have *Brucella* antibodies in the same period as the Arctic foxes were sampled (Table 1). Ingestion of marine mammals has been suggested as the source of infection for seropositive polar bears in this region (Tryland et al. 2001) and in the Beaufort Sea (Atwood et al. 2017).

The fox carcasses in our study, and the blood samples obtained from them had undergone several freeze–thaw cycles. This may have reduced the amounts of antibodies in the samples (Cecchini et al. 1992; Pinsky et al. 2003); it also resulted in heavy haemolysis of the blood in the carcasses prior to sampling, which may hamper some serological tests. However, the iELISA used in the present study has previously shown to be robust when testing samples of similar quality from multiple species (e.g., whales, seals, polar bears and *Rangifer*; Nymo, Godfroid et al. 2013; Nymo, Tryland et al. 2013; Nymo et al. 2018), which is very convenient when working with wildlife. The iELISA is also a multi-species method validated for the detection of s-*Brucella* antibodies in seals, whales, polar bears and *Rangifer* (Nymo, Godfroid et al. 2013), but it is not validated for Arctic foxes. However, the plates were coated with *Brucella abortus* lipopolysaccharides, which is the immunodominant component of s-*Brucella*, shown to cross-react serologically with other s-*Brucella* (Cherwonogrodzky et al. 1990). As conjugate, we used the chimeric protein A/G (Harlow & Lane 1988), which has been used in an iELISA for *Toxoplasma* antibodies in Arctic foxes (Elmore et al. 2016). The most plausible source of infection for the Svalbard Arctic foxes is seals. The iELISA has been used to detect s-*Brucella* antibodies in many different seal species, with results that were supported by other serological methods and bacteriology (Nymo, Godfroid et al. 2013; Nymo et al. 2018). Taking these facts together, we assume that if the investigated Arctic foxes had seroconverted as a result of exposure to s-*Brucella*, the iELISA would have detected s-*Brucella* specific antibodies in spite of poor sample quality.

A potential explanation for the seronegative results could be that the Svalbard Arctic foxes are not susceptible to infection with s-*Brucella*. However, even though Svalbard is an archipelago, the Arctic fox population is not isolated. Satellite tracking of an Arctic fox revealed that it wandered from Svalbard to Ellesmere Island, Canada (Fuglei & Tarroux 2019). Analyses of population genetics have also shown that circumpolar connectivity is maintained amongst Arctic fox populations across most of the species' distribution range, except Iceland (Dalén et al. 2004; Carmichael et al. 2007; Geffen et al. 2007; Noren et al. 2011). It seems unlikely that Arctic foxes in Svalbard would not be susceptible to infection with s-*Brucella*, whereas Arctic foxes in other locations are (Pinigin et al. 1970; Morton 1986, 1989).

The lack of seropositive animals amongst the 406 Arctic foxes tested could theoretically be due *s-Brucella* quickly killing infected foxes, so they were not captured. This also seems unlikely as the marine mammal brucellae are shown to have limited pathological potential in true seals (Phocidae; Nymo et al. 2011) and in cell models (Larsen et al. 2013) and mouse models (Nymo et al. 2016). Moreover, experimental inoculations of red foxes with the pathogenic *B. suis* biovar 4 yielded very limited pathology (Morton 1986, 1989). In addition, no larger disease outbreaks of unknown aetiology were reported in Svalbard Arctic foxes during the study period, although it is challenging to have a full overview of such events in wild populations.

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When screening a large number of true seals, the highest seroprevalence against s-*Brucella* was found in one-year-olds and thereafter declined with age (Nymo et al. 2018). The Arctic foxes in our study were 0–13 years old, thus covering a wide age range and minimizing the odds of missing out a similar epidemiological trait in Arctic foxes. The Arctic foxes investigated also represented geographically distributed locations on the islands of Spitsbergen, Forlandet and Hopen, including numerous coastal locations associated with the marine ecosystem and food chain (Fig. 1).

The lack of s-*Brucella* seropositives amongst the 406 Svalbard Arctic foxes investigated herein is in sharp contrast to a recent screening of Arctic foxes in Iceland, reporting a prevalence of 63 and 25% in coastal and inland Arctic foxes, respectively, suggesting marine mammals as the source of infection (Czirják et al. 2016). The Arctic fox population in Iceland is completely isolated from other Arctic fox populations (Dalén et al. 2004), and s-*Brucella* has not been detected in any other Icelandic terrestrial species (European Centre for Disease Prevention and Control 2019), supporting the suspicion of marine mammals as the reservoir. However, our findings in Svalbard Arctic foxes suggest that marine mammals may not be a major source of infection for this population. Instead, our findings suggest the possibility of an unknown source of s-*Brucella* exposure in Iceland. Polymerase chain reaction amplicon sequences specific for *Brucella* spp. have been detected in common eider (*Somateria mollissima*), common loon (*Gavia immer*), great black-backed gull (*Larus marinus*), great cormorant (*Phalacrocorax carbo*), great shearwater (*Puffinus gravis*), herring gull (*Larus argentatus*) and northern gannet (*Moras bassanus*) from the Canadian–US coast between Kent Island and Virginia (Bogomolni et al. 2008). These birds are present in Iceland and all but the great cormorant and the great shearwater are present in Svalbard and may be part of the Arctic fox diet. Documentation of the ecological range of brucellae has also recently been extended to rodents (Tiller et al. 2010), frogs (Eisenberg et al. 2012), fish (Eisenberg et al. 2017) and soil (Scholz et al. 2008). Whether birds, rodents, fish or the environment is the source of exposure to s-*Brucella* for Arctic foxes in Iceland warrants further investigation.

In the study performed in Iceland by Czirják et al. (2016), the SVANOVIR® *Brucella*-Ab I-ELISA and the serum agglutination tests were utilized, whereas we used a Protein A/G iELISA (Nymo, Godfroid et al. 2013). As the two Arctic fox populations were investigated with different serological methods, the results are not directly comparable. Nonetheless, the large difference in results is surprising. To further explore the contrasting findings from Svalbard and Iceland, additional investigations of Arctic foxes from both places, including a larger serum sample size and organ samples, and using the same serological tests and in addition bacteriological and molecular methods, should be performed. An interesting future avenue would also be to use molecular methods for determining dietary composition, and thus help with identifying potential sources of exposure. Taken together, this could provide interesting information on the epizootiology of *Brucella* spp. in Arctic foxes. If this difference in exposure remains, further investigations are warranted in Iceland in order to identify the source of s-*Brucella* infection in the Arctic foxes there.

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