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Research paper

Seroprevalence for *Brucella* spp. in Baltic ringed seals (*Phoca hispida*) and East Greenland harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals

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ABSTRACT

Zoonotic infections transmitted from marine mammals to humans in the Baltic and European Arctic are of unknown significance, despite given considerable potential for transmission due to local hunt. Here we present results of an initial screening for *Brucella* spp. in Arctic and Baltic seal species. Baltic ringed seals (*Pusa hispida*, $n = 12$) sampled in October 2015 and Greenland Sea harp seals (*Pagophilus groenlandicus*, $n = 6$) and hooded seals (*Cystophora cristata*, $n = 3$) sampled in March 2015 were serologically analysed for antibodies against *Brucella* spp. The serological analyses were performed using the Rose Bengal Test (RBT) followed by a confirmatory testing of RBT-positive samples by a competitive-enzyme linked immunosorbent assay (C-ELISA). Two of the Baltic ringed seals (a juvenile male and a juvenile female) were seropositive thus indicating previous exposure to a *Brucella* spp. The findings indicate that ringed seals in the Baltic ecosystem may be exposed to and possibly infected by *Brucella* spp. No seropositive individuals were detected among the Greenland harp and hooded seals. Although our initial screening shows a zoonotic hazard to Baltic locals, a more in-depth epidemiological investigation is needed in order to determine the human risk associated with this.

1. Introduction

The Baltic and Arctic ecosystems have undergone major change over the past century due to a combination of anthropogenic and natural stressors (Andersen et al., 2010; Jenssen et al., 2015). As is often the case, such changes have been most notably demonstrated by population declines in wildlife species such as harbour seals (*Phoca vitulina*) and hooded seals (*Cystophora cristata*) likely due to phocine distemper virus and PCB exposure causing considerable mortality in past decades (Dietz et al., 1989a, 1989b; Härkönen et al., 2006; ICES, 2011). The significance of infections acting as stressors has likely increased recently as global change facilitates the introduction and spread of new pathogens (Bradley et al., 2005; Greer et al., 2008; Hueffer et al., 2011; Jenkins et al., 2013; Parkinson and Butler 2005; Tryland et al.,

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2013). The increased prevalence of infections is not just of significance for wildlife, it is also an important socioeconomic issue as hunt and tourism is an important activity in the Arctic and Baltic, respectively. In addition, it is wide-spread practice in the Arctic to consume raw meat and internal organs thus introducing an additional human health aspect. The health effect of lack of heat-treatment is exemplified by the seroprevalence for toxoplasmosis, which was 10% within a local Cree population with dietary preference for cooked foods, while it was 80% within Inuit communities consuming raw meat (Lévesque et al., 2007; Messier et al., 2009).

Brucellosis in marine mammals was originally reported in 1994 (Ewalt et al., 1994; Ross et al., 1994). Since then, *Brucella* spp. have been isolated and serotyped in several seal spp. and in walrus (*Odobenus rosmarus*) (Ross et al., 1996; Foster et al., 1996; Nielsen et al., 1996, Jepson et al., 1997, Tryland et al., 1999, Forbes, 2000, Retamal et al., 2000, Nielsen et al., 2001, Van Bressem et al., 2001, Prenger-Berninghoff et al., 2008). *Brucella* infections may cause upper respiratory tract inflammation such as sinusitis as well as more severe conditions such as abortion, infertility, orchitis, bursitis, arthritis and osteomyelitis (Davis et al., 1990; Enright et al., 1990; Ross et al., 1994; Brew et al., 1999). Prior to 1994, marine mammals were not considered to have a host potential for *Brucella* spp. Hereafter two novel *Brucella* spp. were isolated from harbour seals (*Phoca vitulina*) and smaller cetacean spp. (Godfroid et al., 2005; Prenger-Berninghoff et al., 2008; Nymo et al., 2011). In cetaceans, pathology included skin lesions, abscesses, necrosis in the liver and spleen, peritonitis, encephalitis, and spondylitis (Nymo et al., 2011). In harbour seals, *B. pinnipedialis* was most often isolated and associated with bronchopneumonia and septicaemia (Siebert et al., 2017). As with terrestrial mammals including livestock, abortion also play a role in marine mammal infections: reproductive organ pathology and isolation of *Brucella* from aborted foetuses, milk and reproductive organs have been reported in both toothed and baleen whale species (Nymo et al., 2011).

Here we present the serological results for antibodies against *Brucella* spp. in a pilot study of Baltic ringed seals and Greenland harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals.

2. Materials and methods

2.1. Sampling

The geographical distribution of the study populations is shown in Fig. 1. Ringed seal samples (7 juveniles and 5 adults) were obtained during satellite tagging operations in Stora Fjäderägg, the Swedish part of Gulf of Bothnia in October of 2015 (Fig. 1). Seals were caught using commercial monofilament nets (Hvalpsund Nets A/S) and brought to shore in pole nets where they were restrained and sampled for blood. Sex, weight, girth, and length were recorded and individuals were divided into age classes based on their length and weight (Table 1). Blood was drawn from the epidural sinus directly into heparinized vacutainers, and centrifuged at 1100 × *g* for 10 min. The plasma was pipetted off and transferred to cryo-vials that were immediately frozen and stored at −20 °C prior to serological analyses. The project was given ethical and scientific approval by the Swedish Environmental Protection Agency and Umeå Regional Animal Welfare Comittee (permits no. NV-04536-15 and A52-15).

Harp seals (5 adult females and 1 pup) and hooded seals (2 adult females and 1 pup) were sampled for blood in 2015 during a research expedition (The Arctic University of Norway) in the East Greenland pack ice (Fig. 1, Table 1) with the R/V Helmer Hanssen under permits from the Norwegian and Greenland authorities. Captured seals were euthanized in accordance with the Norwegian Animal Welfare Act either by shooting, by intravenous injection of an overdose of barbiturate (30 mg/kg body mass Euthasol vet.; Le Vet B.V., Oudewater, Netherlands) or by complete bleeding in full anaesthesia as described by Geiseler et al. (2016). The project was approved by the National Animal Research Authority of Norway (permits no. 7247, 6216, 5399). Blood was taken from the epidural vein directly into heparinized vacutainers and processed as described above. Biological information for harp and hooded seals are provided in Table 2.

2.2. Serological analyses

Two serological tests were performed to identify *Brucella* spp. antibodies in the plasma. According to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Eloit and Schmitt 2017), the Rose Bengal Test (RBT) is recommended as a general purpose diagnostic test in all wildlife species while the competitive-enzyme linked immunosorbent assay (C-ELISA) appear to be useful for seroepidemiological surveys in wildlife (Stack et al., 1999). Optical density (OD) was assessed at 450 nm using a microplate photometer (air as blank) and the per cent (%) of inhibition (PI) was calculated as:

$$PI = 100 - \frac{(OD\ \ samples\ \ or\ \ control \times 100)}{OD\ \ conjugate\ \ control}$$

Finally, the results were interpreted as negatives (PI < 30%) and positives (PI ≥ 30%).

No specific serological tests for *Brucella* infection in marine mammals have been developed and the detection of specific antibodies is based on tests used for terrestrial mammals (Godfroid 2002). Indirect measures of brucellosis such as antibody tests are in general best supported by the isolation of *Brucella* spp. from individuals in the animal population tested. However, samples other than blood were unavailable for the present study so it was not possible to culture or genotype the specific *Brucella* species. Cross-reactivity in serologic assays between *Brucella* spp. and *Yersenia enterocolitica* is well-documented (Ahvonen et al., 1969; Bundle et al., 1984). However, Tryland et al. (1999) reported no cross reactivity in seals and whales between *Brucella* spp. and *Y. enterocolitica* and they were unable to cultivate *Y. enterocolitica* from any of the tissues from more than 60 marine mammals. Altogether these data strongly suggest that the observed antibody titres in the present study were due to *Brucella* spp. infection.

3. Results and discussion

Two out of the 12 Baltic ringed seals were seropositive in both the RBT and the C-ELISA, indicating that these individuals had been exposed to a *Brucella* spp. Unfortunately, no tissue material was available from the live animals for microbiological analyses. Serological studies of *Brucella* spp. in Baltic ringed seals have not been published previously and our findings indicate that this seal species is actually exposed to *Brucella* bacteria. Our suggestion is supported by a very recent report that a grey seal (*Halichoerus grypus*) in the Baltic Sea screened for *Brucella* spp. were found to be infected by *Brucella pinnipedialis* (Hirvelä-Koski et al., 2017).

All harp ($n = 6$) and hooded ($n = 3$) seals were seronegative. Marine mammal *Brucella* infections are densely distributed in North Atlantic seal and cetacean populations (Jepson et al., 1997; Nielsen et al., 1996; Tryland et al., 1999). In the North-East Barents Sea, anti-*Brucella* antibodies were found in 15 of 811 (2%) harp seals. Further, serosurveys showed a seroprevalence of 15.6% in hooded seals (Nymo et al., 2013), whereas *B. pinnipedialis* was isolated from various organs from 11 of 29 (38%) hooded seals from the pack-ice between Svalbard and Greenland (West Ice) (Tryland et al., 2005). In the study by Nymo et al. (2013) the seropositive individuals were juveniles as in the present study indicating that may this age group is a reservoir for *Brucella*. Persistency, reservoirs and susceptibility have recently been addressed by several studies of *Brucella*. These reports have focused on environmental reservoirs, transmissions and courses and how Brucella may even persistent in macrophages and even fish (Larsen et al., 2016; Nymo et al., 2016a, 2016b).