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.

Key words: Arctic; Humans; One Health; Zoonosis.

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. 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 et al. 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 1990; Enright 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.

Materials and methods

Sampling

The geographical distribution of the study populations is shown in Figure 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 (Figure 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 1100xg for 10 min. The plasma was pipetted off and transferred to cryo-vials that were immediately frozen and stored at –20 ºC prior to serological analyses.

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 (Figure 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.

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 immuno-sorbent 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 that the polar bears in this study had been exposed to and mounted a humoral immune response against. 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. In another study from Alaska, O'Hara et al. (2010) showed that *Brucella* spp. found in Alaskan polar bears were likely to be of terrestrial and not marine origin. Altogether these data strongly suggest that the observed antibody titres in polar bears in the present study were due to *Brucella* spp. infection.

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 a 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).

In contrast, no anti-*Brucella* antibodies were detected in ringed seals (*n* = 20) from Svalbard (Tryland et al. 2005). The finding that none of the harp and hooded seals in the present study were seropositive for *Brucella* could be a sole effect of the low sample size. Harp seal investigations conducted by Maratea et al. (2003) of stranded animals on Rhode Island showed similar results. Differences in exposure levels as reflected in seroprevalence may exist, but larger more comprehensive epidemiological studies are needed for firm conclusions. However, *Brucella* infections should be considered as an important infection of seals in the northern Baltic Sea area and East Greenland. Accordingly, the CRC Marine Mammal Handbook and other publications have deemed *Brucella* as the most significant emerging bacterial zoonosis in pinnipeds (Miller et al. 2001; Ross et al. 1996; Tryland et al. 2013).

Due the handling of hunted seals and digestion of raw seal tissues, East Greenland hunters are at a particular risk being exposed to seal-associated *Brucella*. Human brucellosis cases have been reported but fatal infections have not yet been diagnosed (Sohn et al. 2003; McDonald et al. 2006; Brew et al. 1999). However, it has been estimated that only 10% of *Brucella* infections in humans are diagnosed, which is partly due to its unspecific clinical signs and disease progression (Brew et al. 1999; Hernández-Mora et al. 2013).

In addition to the zoonotic implications of brucellosis, this infection may also have significant impact on population management and sustainability of seal harvest and quotas. Further studies are needed to address the abortifacient potential of *Brucella*-infections in seals as well as the reservoirs, routes of transmission, course of infection and the pathogenicity and impact for different seal species and populations. This should be investigated concurrently with the drastic environmental changes in the Baltic and Arctic over the past decades (Andersen et al. 2010; Dietz et al. 1989a; 1998b; Härkönen et al. 2006; Roos et al. 2012), which likely act in concert to influence the health of the ecosystems and their constituent species.

Conclusions

This study adds weight of evidence to the prevalence of *Brucella* antibodies in Baltic and Arctic seal species. These two ecosystems are already exposed to natural and anthropogenic stressors and the infection biology of *Brucella* infections in seals needs to be established to better understand seal population dynamics. Furthermore, people in the Baltic that handle ringed seals may be exposed to Brucella infections and further investigations on this zoonotic potential is warranted.

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TABLES

Table 1. Information of the East Greenland harp seals (*n* = 6) and hooded seals (*n* = 3) collected 21-27 March 2015. SL: standard length. BW: body weight. Due to missing log-book during field work some data are missing.

ID	Species	Sex	Age group	SL (cm)	BW (kg)	Date	Position	Serostatus
H1	Harp seal	Female	Adult	176	136	21-03-2015	72 49'N, 14 19'W	Negative
H2	Harp seal	Female	Adult	156	104	22-03-2015	72 19'N, 14 59'W	Negative
H3	Harp seal	Female	Adult		110	22-03-2015	72 19'N, 14 59'W	Negative
H4	Harp seal	Female	Adult	173	127	24-03-2015	71 41'N, 16 38'W	Negative
H4	Harp seal		Pup			24-03-2015	71 41'N, 16 38'W	Negative
H5	Harp seal	Female	Adult	166	82	28-03-2015	70 47'N, 18 46'W	Negative
K2	Hooded seal	Female	Adult		154	23-03-2015	71 53'N, 15 44'W	Negative
K4	Hooded seal		Pup			23-03-2015	71 53'N, 15 52' W	Negative
K7	Hooded seal	Female	Adult		174	27-03-2015	71 12'N, 18 11'W	Negative

Table 2. Biological information of the Baltic ringed seals ($n = 12$) sampled in Sweden on 15 October 2015. SL: standard length. BW: body weight.

ID	**Sex**	**Age group**	**SL (cm)**	**BW (kg)**	**Serostatus**
VS 15-01	Male	Juvenile	85	32.5	Negative
VS 15-02	Male	Juvenile	89	33.0	**Positive**
VS 15-03	Male	Juvenile	87	32.5	Negative
VS 15-04	Male	Adult	116	56.5	Negative
VS 15-05	Male	Adult	105	62.0	Negative
VS 15-06	Female	Juvenile	99	49.5	Negative
VS 15-07	Male	Adult	115	84.5	Negative
VS 15-08	Male	Adult	118	72.0	Negative
VS 15-09	Female	Juvenile	99	34.0	**Positive**
VS 15-10	Female	Juvenile	97	36.0	Negative
VS 15-11	Female	Adult	107	53.0	Negative
VS 15-12	Female	Juvenile	91	30.5	Negative

FIGURE LEGENDS

Figure 1. Map showing the sample sites for the Baltic ringed seals and East Greenland harp and hooded seals included in the present study.

FIGURES

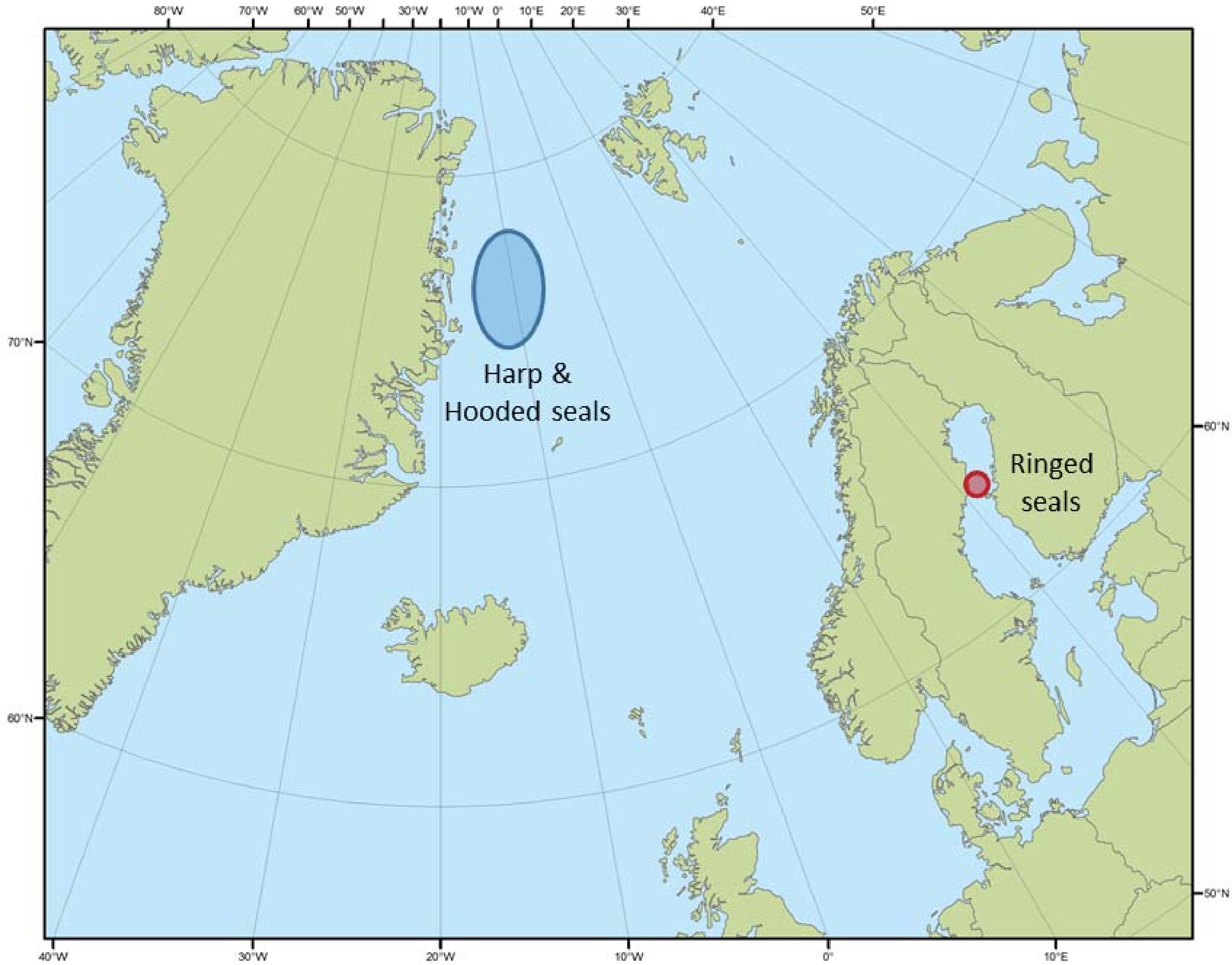

FIGURE 1