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-Berntinghoff 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:

\[
PI = 100 - \left(\frac{OD \text{ samples or control}}{OD \text{ conjugate control}} \times 100\right)
\]

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 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).

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;

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.

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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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.

<table>
<thead>
<tr>
<th>ID</th>
<th>Species</th>
<th>Sex</th>
<th>Age group</th>
<th>SL (cm)</th>
<th>BW (kg)</th>
<th>Date</th>
<th>Position</th>
<th>Serostatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Harp seal</td>
<td>Female</td>
<td>Adult</td>
<td>176</td>
<td>136</td>
<td>21-03-2015</td>
<td>72 49'N, 14 19'W</td>
<td>Negative</td>
</tr>
<tr>
<td>H2</td>
<td>Harp seal</td>
<td>Female</td>
<td>Adult</td>
<td>156</td>
<td>104</td>
<td>22-03-2015</td>
<td>72 19'N, 14 59'W</td>
<td>Negative</td>
</tr>
<tr>
<td>H3</td>
<td>Harp seal</td>
<td>Female</td>
<td>Adult</td>
<td>110</td>
<td>127</td>
<td>24-03-2015</td>
<td>71 41'N, 16 38'W</td>
<td>Negative</td>
</tr>
<tr>
<td>H4</td>
<td>Harp seal</td>
<td>Female</td>
<td>Pup</td>
<td>173</td>
<td>82</td>
<td>28-03-2015</td>
<td>70 47'N, 18 46'W</td>
<td>Negative</td>
</tr>
<tr>
<td>H5</td>
<td>Harp seal</td>
<td>Female</td>
<td>Adult</td>
<td>166</td>
<td>154</td>
<td>23-03-2015</td>
<td>71 53'N, 15 44'W</td>
<td>Negative</td>
</tr>
<tr>
<td>K2</td>
<td>Hooded seal</td>
<td>Female</td>
<td>Adult</td>
<td>23-03-2015</td>
<td>71 53'N, 15 52' W</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K4</td>
<td>Hooded seal</td>
<td>Pup</td>
<td></td>
<td>27-03-2015</td>
<td>71 12'N, 18 11'W</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Table 2.** Biological information of the Baltic ringed seals ($n = 12$) sampled in Sweden on 15 October 2015. SL: standard length. BW: body weight.

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age group</th>
<th>SL (cm)</th>
<th>BW (kg)</th>
<th>Serostatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS 15-01</td>
<td>Male</td>
<td>Juvenile</td>
<td>85</td>
<td>32.5</td>
<td>Negative</td>
</tr>
<tr>
<td>VS 15-02</td>
<td>Male</td>
<td>Juvenile</td>
<td>89</td>
<td>33.0</td>
<td>Positive</td>
</tr>
<tr>
<td>VS 15-03</td>
<td>Male</td>
<td>Juvenile</td>
<td>87</td>
<td>32.5</td>
<td>Negative</td>
</tr>
<tr>
<td>VS 15-04</td>
<td>Male</td>
<td>Adult</td>
<td>116</td>
<td>56.5</td>
<td>Negative</td>
</tr>
<tr>
<td>VS 15-05</td>
<td>Male</td>
<td>Adult</td>
<td>105</td>
<td>62.0</td>
<td>Negative</td>
</tr>
<tr>
<td>VS 15-06</td>
<td>Female</td>
<td>Juvenile</td>
<td>99</td>
<td>49.5</td>
<td>Negative</td>
</tr>
<tr>
<td>VS 15-07</td>
<td>Male</td>
<td>Adult</td>
<td>115</td>
<td>84.5</td>
<td>Negative</td>
</tr>
<tr>
<td>VS 15-08</td>
<td>Male</td>
<td>Adult</td>
<td>118</td>
<td>72.0</td>
<td>Negative</td>
</tr>
<tr>
<td>VS 15-09</td>
<td>Female</td>
<td>Juvenile</td>
<td>99</td>
<td>34.0</td>
<td>Positive</td>
</tr>
<tr>
<td>VS 15-10</td>
<td>Female</td>
<td>Juvenile</td>
<td>97</td>
<td>36.0</td>
<td>Negative</td>
</tr>
<tr>
<td>VS 15-11</td>
<td>Female</td>
<td>Adult</td>
<td>107</td>
<td>53.0</td>
<td>Negative</td>
</tr>
<tr>
<td>VS 15-12</td>
<td>Female</td>
<td>Juvenile</td>
<td>91</td>
<td>30.5</td>
<td>Negative</td>
</tr>
</tbody>
</table>
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.
Harp & Hooded seals

Ringed seals