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Age-dependent prevalence of anti-*Brucella* antibodies in hooded seals *Cystophora cristata*

Ingebjørg H. Nymo[1,*,], Morten Tryland[1,**], Anne Kirstine Frie[2,**], Tore Haug[2,**], Geoffrey Foster[3], Rolf Rødven[4,**], Jacques Godfroid[1,**]**

[1]Section of Arctic Veterinary Medicine, Norwegian School of Veterinary Science, Stakkevollveien 23, 9010 Tromsø, Norway
[2]Institute of Marine Research, PO Box 6404, 9294 Tromsø, Norway
[3]SAC Consulting Veterinary Services, Drummondhill, Stratherrick Road, Inverness IV2 4JZ, UK
[4]Bioforsk — Norwegian Institute of Agricultural and Environmental Research, PO Box 2284, 9269 Tromsø, Norway

ABSTRACT: Investigations of hooded seals *Cystophora cristata* have revealed high prevalences of *Brucella*-positive seals in the reduced Northeast Atlantic stock, compared to the increasing Northwest Atlantic stock. This study evaluated the relation between *Brucella*-serostatus in seals in the Northeast Atlantic stock and age, sex, body condition and reproduction. Bacteriology documented which animals and organs were *B. pinnipedialis* positive. No relationship was observed between *Brucella*-serostatus and body condition or reproductive traits. Pups (<1 mo old) had a substantially lower probability of being seropositive (4/159, 2.5%) than yearlings (6/17, 35.3%), suggesting that exposure may occur post-weaning, during the first year of life. For seals >1 yr old, the mean probability of being seropositive decreased with age, with no seropositives older than 5 yr, indicating loss of antibody titre with either chronicity or clearance of infection. The latter explanation seems to be most likely as *B. pinnipedialis* has never been isolated from a hooded seal >18 mo old, which is consistent with findings in this study; *B. pinnipedialis* was isolated from the retropharyngeal lymph node in 1 seropositive yearling (1/21, 5%). We hypothesize that this serological and bacteriological pattern is due to environmental exposure to *B. pinnipedialis* early in life, with a subsequent clearance of infection. This raises the question of a reservoir of *B. pinnipedialis* in the hooded seal food web.

KEY WORDS: Pinniped · Pups · Brucellosis · Serostatus · Bacteriology · Infection clearance · Atlantic hooded seal stock · Food web

INTRODUCTION

Two management stocks of hooded seals *Cystophora cristata* are distributed throughout much of the North Atlantic and adjacent Arctic Ocean (Kovacs 2009): the Northwest Atlantic stock (NWS) and the Northeast Atlantic stock (NES) (Fig. 1). The NWS whelps off the east coast of Newfoundland, in the Gulf of St Lawrence and in the Davis Strait, whereas the NES whelps off the east coast of Greenland, slightly north of the island Jan Mayen (the West Ice) (Rasmussen 1957, Sergeant 1974, Kovacs 2009). The 2 stocks moult on the pack-ice in the Denmark Strait (NWS) and in the West Ice (NES) in June/July (Kovacs 2009). Satellite data indicate fidelity to their breeding areas (Hammill 1993, Folkow et al. 1996), and the stocks show separation in movements and population dynamics (Folkow et al. 1996, 2010, Andersen et al. 2009). Interaction between the 2 stocks is, however, registered (Andersen et al. 2009), and they cannot be separated with genetic analyses (Coltman et al. 2007). Outside the breeding and moulting periods they perform long feeding excursions to the Arctic waters between Canada and

*Email: ingebjorg.nymo@nvh.no
**Member of the Fram centre–High North Research Centre for Climate and the Environment, 9296 Tromsø, Norway

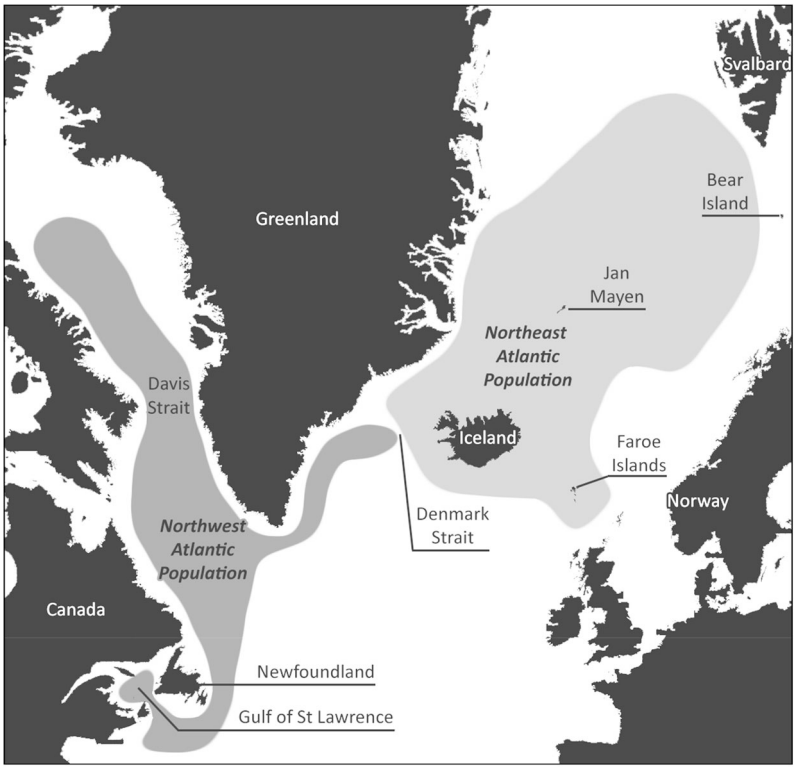

Fig. 1. *Cystophora cristata*. Distribution of hooded seals from the Northwest Atlantic (darker grey) and Northeast Atlantic (lighter grey) populations

Greenland (NWS) (Andersen et al. 2009) and the Nordic Seas (Greenland, Norwegian and Icelandic Seas, NES) (Folkow et al. 1996, 2010).

While estimates of abundance in the NWS have increased since the 1980s (Hammill & Stenson 2006), abundance of the NES appears to have decreased to only 10 to 15% of the 1946 population size, from 575 000 hooded seals in 1946 to approximately 85 000 hooded seals in 2011, and has remained stable at this low level since the 1980s (ICES 2011). Hooded seal hunts in the West Ice have been conducted since the 18th century, and after 1920 the hunt was of a significant extent. In 1958, agreements were made between Norway and the Soviet Union on time and activity restrictions on the hooded seal hunt in the West Ice, but it was not until 1971 that quotas were introduced (Bjørge 2010). The introduction of quotas did not alter the negative population development in the NES, and due to the decline of the NES, no commercial hunt has been conducted on the NES since 2007 (ICES 2011), and the hooded seal species has been classified since 2008 as 'Vulnerable' in the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) (Kovacs 2008).

Brucella spp. were first isolated from marine mammals in 1994 (Ross et al. 1994) and were validly published as new species within the genus *Brucella* as *B. pinnipedialis* and *B. ceti* in 2007 (Foster et al. 2007), with pinnipeds and cetaceans as preferred hosts, respectively. Pathological changes, including lesions of the reproductive organs and associated abortions, have only been registered in cetaceans (Foster et al. 2002, Nymo et al. 2011, Guzmán-Verri et al. 2012). The marine mammal *Brucella* spp. have shown variation in key aspects of virulence (Maquart et al. 2009a); the ability to survive and multiply in macrophages (Gorvel & Moreno 2002), and different clusters within the *B. ceti* and *B. pinnipedialis* species have been recognized according to their preferred host, bacteriological properties and distinct genetic characteristics (Whatmore et al. 2007, Maquart et al. 2009b, Guzmán-Verri et al. 2012). There are indications that certain marine mammal *Brucella* spp. have zoonotic potential; 3 human cases have been reported, all with neurobrucellosis, but no known contact with marine mammals, and all had a history of exposure to raw products from the ocean (Sohn et al. 2003, McDonald et al. 2006). The 3 strains were of an unusual sequence (Type 27) only recently recovered from a bottlenose dolphin foetus from a region where few marine mammal isolates have been investigated (Whatmore et al. 2008).

The 2 hooded seal stocks show a remarkable difference in prevalence of anti-*Brucella* antibodies: 31 to 35% in the decreased NES (Tryland et al. 1999, 2005) versus 5% in the increasing NWS (Nielsen et al. 2001). Investigation of macroscopically healthy hooded seals from the NES led to isolation of *B. pinnipedialis* from numerous organs in 38% of the individuals examined (Tryland et al. 2005), while a bacteriological investigation targeting *B. pinnipedialis* in hooded seals from the NWS has not been performed. *B. pinnipedialis* has also been isolated from various organs in 3 young hooded seals, 5 to 9 mo of age, with no *Brucella*-associated pathology, stranded on the coast of Scotland (Foster et al. 1996, 2002).

Juvenile growth and survival, and onset of reproduction are fitness components strongly influencing

the population dynamics of mammals (Gaillard et al. 2000, Herrando-Pérez et al. 2012). In general individuals of poor condition are more susceptible to infections, some of which may further reduce condition and, hence, increase susceptibility (Beldomenico et al. 2008). Little is known, however, about how fitness traits influence the susceptibility to, and impact of, *B. pinnipedialis* infections.

The aim of the present study was to evaluate how *Brucella*-serostatus in hooded seals in the NES was related to age, sex and body condition. We also investigated how traits directly linked to fitness were related to *Brucella*-serostatus, e.g. the presence of corpora lutea (CL) and corpora albicantia (CA), as well as body size and the condition of pups and yearlings. Bacteriological investigations of organ samples from 21 hooded seals were performed to address at which age and in which organs *B. pinnipedialis* was present.

MATERIALS AND METHODS

Samples

The hooded seals *Cystophora cristata* were killed according to Norwegian law (Fiskeri- og kystdepartementet 2003). Samples from the NES (71 to 78° N, 2 to 18° W) were obtained during scientific hunts in 1999 (September/October), 2004 (April/May), 2008 (July), 2009 (April/May) and 2010 (July), as a part of a project (Institute of Marine Research, Tromsø, Norway) approved by the Norwegian government, addressing the ecology and health status of the NES (ICES 2011). All hooded seals included in the study were categorized as pups (<1 mo, n = 159) or adults (≥1 yr, n = 220). The pups were captured either while still with their mother, or alone on the pack-ice. All exhibited the blueback coat, i.e. a blue-grey color dorsally and a pale cream color ventrally (Kovacs 2009). The yearlings may sometimes harbour the blueback coat, but they can be recognized because they are longer and have a thinner blubber layer. For a subsample of adult females (1 to 18 yr old, n = 92), the exact age in years was determined by counting annual growth layer groups in teeth (Frie et al. 2012) (Table S1 in the Supplement at www.int-res.com/articles/suppl/d106p187.pdf). Body length in centimetres (dorsal midline from tip of nose to tip of tail), weight in kilograms and dorsal blubber thickness in millimetres (dorsal midline, right behind the front flippers) were measured.

The reproductive status of females was assessed by the presence of CL and CA according to Frie et al. (2012). Blood (n = 379: 164 males, 212 females, 3 of unknown sex, Table 1) was collected in evacuated blood containers (Venoject®, Terumo) from vena and arteria brachialis during terminal bleeding of the seals, and left standing overnight at room temperature. The serum was collected (1000 × *g*, 10 min) and stored at –20°C. Organ samples from 21 hooded seals (Table 2) were obtained aseptically within 30 min post mortem and stored at –20°C in sealed, sterile plastic bags. Samples included liver (n = 21), spleen

Table 1. *Cystophora cristata*. Summary of serum samples included in the study from the Northeast Atlantic stock of hooded seals per year, divided into pups and adults. Numbers of females are given first, males are given second and animals with unknown sex are given last

Year	Pups	Adults
1999	4/4/2	11/0/0
2004	4/3/0	15/0/0
2008	0/0/0	20/13/0
2009	69/73/0	6/5/0
2010	0/0/0	83/66/1

Table 2. *Cystophora cristata*. Age, sex, *Brucella*-serostatus and bacteriological status (+: positive; –: negative) of hooded seals from the Northeast Atlantic stock included in the bacteriological investigation. Sampling year is set as the second part of the animal identity number (09: 2009; 10: 2010). A: adult (≥1 yr); F: female; M: male

Animal ID no.	Age (yr)	Sex	Serology	Bacteriology
117/09	0	F	+	–
116/09	0	M	+	–
03/10	1	F	+	+
07/10	1	F	+	–
02/10	1	F	–	–
58/10	2	F	–	–
41/10	3	F	+	–
59/10	3	F	+	–
44/10	3	F	–	–
93/10	3	F	–	–
53/10	4	F	–	–
61/10	4	F	–	–
64/10	4	F	–	–
96/10	4	F	–	–
102/10	4	F	–	–
91/10	11	F	–	–
49/10	12	F	–	–
01/10	A	M	+	–
06/10	A	M	+	–
08/10	A	M	+	–
99/10	A	M	+	–

(n = 21), kidney (n = 21), lung (n = 19), muscle (n = 19), testes (n = 4), mesenteric lymph node (n = 20), mediastinal lymph node (n = 19), retropharyngeal lymph node (n = 15), inguinal lymph node (n = 7), mammary lymph node (n = 5) and internal iliac lymph node (n = 2).

Serology

Serum samples were analysed for anti-*Brucella* antibodies with a Protein A/G indirect enzyme-linked immunosorbent assay (ELISA) for the detection of anti-*Brucella* antibodies in hooded seals (Nymo et al. 2013).

Statistical analysis

After checking for non-linearity using additive models (Wood 2006), the relation between *Brucella*-serostatus (0/1), age, sex and body condition was analyzed using generalized linear models (function glm in R library base). A logistic regression with a binomial error structure and a logit link was assumed. Seroprevalence followed a non-linear pattern with increasing age, due to an abrupt increase from pups to yearlings, and data were therefore analyzed in 2 steps. First, seroprevalence was compared between the age groups pups (<1 mo) and adults (≥1 yr); thereafter seroprevalence was investigated for a subsample of the adults (≥1 yr) with tooth-based age estimated.

For the pup group (<1 mo) versus the adult group (≥1 yr) the full model included age group (pups/adults), sex and dorsal blubber thickness, as well as the interaction between age group and the latter 2 parameters. One 7 yr old female caught in 2010 was regarded as an outlier and hence removed from the dataset, as the dorsal blubber thickness of this individual was about 4 times the average dorsal blubber thickness of individuals of the same age, thus strongly indicating a possible measurement error.

As the estimated tooth-based age was highly correlated with body mass (r = 0.87, CI = [0.84, 0.90]) and body length (r = 0.84, CI = [0.80, 0.88]) for adults, but less so with dorsal blubber thickness (r= −0.46, CI = [−0.55, −0.35]), the latter was used as a proxy for body condition to reduce colinearity in the statistical model. The full model for adults thus included estimated tooth-based age, sex, dorsal blubber thickness and the interaction between estimated tooth-based age and the latter 2 parameters.

For adults, the relation between *Brucella*-serostatus and fitness-related traits was assessed by investigating *Brucella*-serostatus and the presence of CL or CA separately, with binomial models with a logit link. For the total number of CA a Poisson distribution was assumed. CLs were only variably present in individuals aged 2 to 4 yr, while the respective age interval was 4 to 6 yr for CA; CL and CA were absent in individuals below these ages and present in all individuals above these ranges. Thus, analyses were restricted to the age intervals 2 to 4 yr for CLs (n = 67) and 4 to 6 yr for CAs (n = 53). The interaction between *Brucella*-serostatus, age and dorsal blubber thickness was included in the models to allow for variable susceptibility at different ages and different body conditions.

For pups and yearlings, the relation between *Brucella*-serostatus and body size (body length, weight and dorsal blubber thickness, including sex and year as covariates) was investigated using linear models (function lm in R library base), assuming that early growth may influence subsequent fitness indirectly through size-dependent survival or onset of reproduction (Lindström 1999). This was not investigated for hooded seals that were 1 yr and older, since survival in general is less dependent on body size (Gaillard et al. 2000) and because reproductive status was assessed directly by investigating the presence of CL or CA.

Exposure and susceptibility may vary among years for adults. As we only had tooth-based age estimates for female adults from 2010 (Table S1), age and year could not be included in the same model; however, testing differences in the likelihood of adults being *Brucella*-seropositive among years showed no significant differences (ΔAIC_c [Akaike's information criterion corrected for small sample size] > 2.7, 95% CI: 2004: [−3.84, 0.12]; 2008: [−2.07, 0.78]; 2009: [−2.69, 0.95]; 2010: [−2.26, 0.29], 1999 as the reference level, n = 221), suggesting that differing age structure among years did not confound the influence of age on seroprevalence.

The final models were selected using ΔAIC_c (AIC_c; Akaike 1974). In cases where the difference in AIC_c was <1, the most parsimonious model was selected (for details on model selection, please see Tables S2 to S12 in the Supplement) (Burnham & Anderson 2002). Predictors were considered significant when their 95% CI did not include zero (estimated by the function *confint* in R library base for linear models). Statistical analyses were conducted using the software R, Version 2.14.1.

Bacteriology

Organ samples were inoculated on Columbia sheep blood agar (Oxoid) and Farrell s medium (Foster et al. 1996) and incubated at 37°C in an atmosphere of air plus 5% CO_2 for up to 14 d. Colonies typical of *Brucella* spp. were sub-cultured on Columbia sheep blood agar and incubated as before, and also in air without CO_2 supplement to determine whether the isolates were CO_2-dependent. Isolates were further examined by Gram-stain, modified Ziehl-Nielsen stain (Alton et al. 1988) and slide-agglutination with a *B. abortus* antiserum (Remel Europe). The isolates were identified by growth characteristics, agglutination with monospecific sera and phage typing at the FAO/WHO Collaborating Centre for Brucellosis at the Veterinary Laboratories Agency, Weybridge, UK.

RESULTS

Serology

The overall seroprevalence in *Cystophora cristata* was 15.6% (59/379): 16.5% (27/164) in males and 15.1% (32/212) in females.

First, seroprevalence was compared between the age groups pups (<1 mo) and adults (≥1 yr). Animals in the pup group had a lower probability of being seropositive than animals in the adult group (2.5%, 4/159, 95% CI = [0.8, 5.7] versus 25.0%, 55/220, 95% CI = [19.6, 31.0]). Sex (ΔAIC_c > 1.1) and dorsal blubber thickness (ΔAIC_c > 1.0), or interaction terms (ΔAIC_c > 1.7), were not included in the final model (Tables 3 & S2 in the Supplement).

Seroprevalence was also investigated for a subsample of the adults (≥1 yr) with tooth-based age estimated (n = 92) (Table S3 in the Supplement). The main reason for the increase in seroprevalence from the pup group to the adult group was a high probability of being seropositive for yearlings (35.3%, 6/17, 95% CI = [15.8, 58.9]) (Table 3). The mean probability of being seropositive decreased with age for hooded seals >1 yr, (Table 3, Fig. 2), and all seropositive adults (≥1 yr) were 1 to 5 yr old (Fig. 2). Dorsal blubber thickness was included in the final model, but the relation barely differed from zero (95% CI for slope: [−0.19, −0.00]) and was strongly influenced by 1 individual; therefore, its biological significance was considered as negligible. Sex was not included in the final model (ΔAIC_c > 2.0).

Table 3. *Cystophora cristata*. Relation between *Brucella*-serostatus and age groups (adults ≥1 yr versus pups <1 mo) or age (1 to 18 yr old) for hooded seals from the Northeast Atlantic stock. Estimates are given as logit-transformed probabilities for the final models. For the predictor age group, pups were used as the reference level, i.e. the estimate shows the difference compared to pups

Response	Predictor	Estimate	SE	95% CI
Seropositive (logit(Prob)) (n = 378)	Intercept	−3.66	0.51	[−4.84, −2.80]
	Age group (pups vs. adults)	2.56	0.53	[1.64, 3.77]
Seropositive (logit(Prob)) (n = 91)	Intercept	3.00	1.45	[0.32, 6.07]
	Age (1–18 yr)	−0.74	0.24	[−1.28, −0.32]
	Dorsal blubber	−0.08	0.05	[−0.19, −0.00]

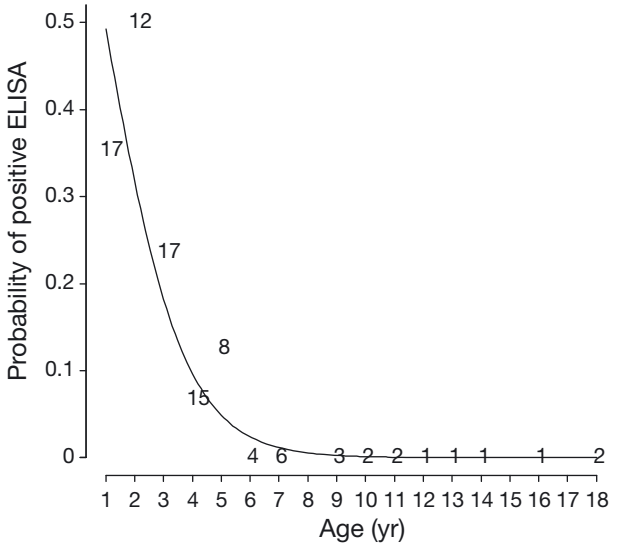

Fig. 2. *Cystophora cristata*. Predicted mean influence of age for adult hooded seals (1 to 18 yr old) from the Northeast Atlantic stock on the probability of being *Brucella*-seropositive (solid line), adjusted for the influence of mean dorsal blubber thickness (Table 3). Numbers indicate the sample sizes at different ages, while their locations show the empirical probabilities

There was no relationship observed between *Brucella*-serostatus in pups and weight ($\Delta AICc$ > 1.4), length (ΔAIC_c > 2.1) or dorsal blubber thickness (ΔAIC_c > 0.41) (Tables S4 to S6 in the Supplement). For yearlings there was no relation between *Brucella*-serostatus and weight (ΔAIC_c > 0.8), length (ΔAIC_c > 2.3) or dorsal blubber thickness (ΔAIC_c > 3.0) (Tables S7 to S9 in the Supplement). *Brucella*-serostatus was not related to the presence of CL (ΔAIC_c > 2.2) or CA (ΔAIC_c > 2.7), or the number of CAs for females 4 to 6 yr of age (ΔAIC_c > 2.2) (Tables S10 to S12 in the Supplement).

Bacteriology

There were no signs of gross pathology in any of the organs investigated. *Brucella pinnipedialis* was isolated from 1 out of 21 animals investigated. The positive sample yielded a single colony from the retropharyngeal lymph node of a seropositive, 1 yr old female (Table 2). The bacteria were Gram-negative cocco-bacilli that were acid-fast when stained with the modified Ziehl-Neelsen method. The isolate agglutinated with *B. abortus* antiserum in slide tests, required increased CO_2 for growth, was urease, catalase and oxidase positive, did not produce H_2S, and grew in the presence of basic fuchsin (20 µl ml^{-1}) and thionin (20 µl ml^{-1}). The isolate was agglutinated by the A monospecific antiserum and not by the M monospecific antiserum. The isolate was lysed at the routine test dilution with the phages Wb, Tb, BK_2, R/C, Fi and Iz.

DISCUSSION

To our knowledge this is the first report investigating the relation between *Brucella*-serostatus in hooded seals *Cystophora cristata* in the NES and age, sex, body condition and reproductive traits. An age-dependent serological pattern was identified with a low probability of being seropositive for the pup group (<1 mo) and a high probability for yearlings, suggesting exposure post-weaning, during the first year of life. For seals >1 yr of age, the mean probability of being seropositive decreased with age, with no seropositives older than 5 yr. These findings could be explained by a loss of antibody titre during a chronic infection, or clearance of infection, the latter seeming most likely as *B. pinnipedialis* has never been isolated from a hooded seal >18 mo. We hypothesize that this particular serological and bacteriological pattern could be due to environmental exposure to *B. pinnipedialis* post-weaning, during the first year of life, with a subsequent clearance of infection.

We isolated *Brucella pinnipedialis* from the retropharyngeal lymph node of a seropositive 1 yr old, with no associated gross pathology. The bacteriology characteristics were in line with previous findings (Dawson et al. 2008), except that our isolate showed lysis with the R/C phage described as active against rough strains of *Brucella* spp. (Alton et al. 1988). The isolate was A+M–, like the 3 Scottish hooded seal isolates (Foster et al. 1996, 2002), but in contrast to the isolates described by Tryland et al. (2005), which were A+M+. The presence of *B. pinnipedialis* in 1 out of 21 (5%) of the hooded seals in this study was in contrast to the 38% (11/29) bacteriology positive hooded seals previously found in the NES. It is important to note, however, that in the previous study all hooded seals positive according to bacteriology were 6 to 18 mo of age (Tryland et al. 2005), whereas in the present study only 6 of the 21 seals tested bacteriologically were ≤2 yr old (Table 2). The difference in prevalence might therefore reflect the age composition of the tested animals.

In the 2 previous *Brucella* serological studies of the NES, the majority (age was not estimated for all) of the hooded seals were between 1 and 4 yr old (Tryland et al. 1999) and between 6 and 18 mo old (23 out of 29) (Tryland et al. 2005). In contrast, in the study performed in the NWS the majority of the animals were >3 (females) or >4 (males) yr old (168 out of 204) (Nielsen et al. 2001). If the same age-dependent serological pattern is present in both stocks, the difference in seroprevalence between the stocks could be due to the age composition of the animals tested. Further investigations are warranted in order to draw final conclusions.

Body size and condition of pups and yearlings are indirectly linked to fitness through their influence on survival and onset of reproduction (Lindström 1999), and there was no relation between serostatus and body size in pups or yearlings. We found no relation between *Brucella*-serostatus and presence of CL and CA in young females either. While these factors are sensitive to environmental constraints (Gaillard et al. 2000, Herrando-Pérez et al. 2012), our findings are consistent with the lack of *Brucella*-associated lesions when isolating *B. pinnipedialis* from pinnipeds (Nymo et al. 2011). Prior investigation of aborted and premature Californian sea lions *Zalophus californianus* showed the presence of *Brucella* spp. DNA in 3 out of 59 placentae, and 2 of the placentae were also culture positive, but had no *Brucella*-associated histopathology (Goldstein et al. 2009). Abortions are also commonly observed in Australian fur seals *Arctocephalus pusillus*, and a high prevalence of *Brucella*-seropositive adult females (57%) has been detected (Lynch et al. 2011a), while the seroprevalence among pups was close to 0%. Pathology seen in 4 aborted foetuses was consistent with bacterial infection, but *B. pinnipedialis* could not be detected by bacteriology or PCR in the aborted fetuses or in the placentae (Lynch et al. 2011b). In contrast, *B. ceti* has been isolated from lesions in the uterus and testes, and from aborted foetuses, vaginal secretions, milk and mammary glands in cetaceans (Guzmán-Verri et al. 2012),

indicating that *B. ceti* is causing pathology similar to what is typical for brucellosis in terrestrial mammals (Corbel & Brinley-Morgan 1984). Thus, while clinical brucellosis has been documented in cetaceans, reports of the presence of *B. pinnipedialis* in a wide range of organs in seals have not been associated with such pathology (Nymo et al. 2011, Guzmán-Verri et al. 2012). Presence of CL and CA are used to estimate age at sexual maturity and fertility rates of hooded seals (Frie et al. 2012); however, an abortion in late pregnancy or a premature birth, as commonly seen with *Brucella*-induced abortion in terrestrial animals (Corbel & Brinley-Morgan 1984), might not affect the presence of CL. Thus, although our results show no relation between *Brucella*-serostatus and ovulation rate or neo-natal body condition, an influence of *B. pinnipedialis* on late abortion or neo-natal mortality cannot be excluded based on the data available here.

Seals, as dogs, have an endotheliochorial placenta (Stewart & Stewart 2009). In species with this placenta type, 5 to 10% of the maternal antibodies are transferred *in utero*, while the majority of them are transferred through the colostrum. The immunity transmitted by the colostrum is determined by the level of systemic immunity of the mother (Tizard 2000), and litters of pups from *Brucella canis*-infected bitches have antibodies for *B. canis* (Carmichael & Kenney 1970). The low number of seropositive pups in this study indicates that they are not receiving maternal antibodies against *B. pinnipedialis*. This is consistent with the finding that most hooded seals no longer have a detectable level of anti-*Brucella* antibodies by the time they give birth; the mean age at primiparity is estimated to be 5.7 yr (ICES 2011), and indeed we found no seropositive seals >5 yr of age.

One of the hallmarks of *Brucella* spp. is the ability to establish chronic infections (von Bargen et al. 2012). For hooded seals >1 yr, the mean probability of being seropositive decreased with age, with no seropositives >5 yr of age. This finding could indicate that hooded seals, like other species susceptible to *Brucella*-infection, lose their antibody titers when entering a chronic state of disease (Grilló et al. 1999, Godfroid et al. 2010). However, the concurrent lack of isolation from hooded seals >18 mo (Foster et al. 1996, 2002, Tryland et al. 2005) indicates clearance of infection and loss of antibody titre with time (Abbas et al. 2010). An alternative explanation to the reduced mean probability of being seropositive with increasing age could be mortality in juveniles due to *B. pinnipedialis*, but this seems unlikely as *B. pinnipedialis* has never been isolated in relation to any pathology in pinnipeds (Nymo et al. 2011).

The mean probability of being seropositive increased from pups to yearlings, indicating exposure to *Brucella pinnipedialis* post-weaning, during the first year of life. The hooded seal pup is born in an advanced state, and the lactation period lasts for only 3 to 5 d (Kovacs 2009). The pups leave the pack-ice at approximately 1 mo of age and lead a solitary pelagic life for the subsequent months, visiting distant foraging areas (Folkow et al. 2010), resembling the pattern of adults (Folkow et al. 1996). The diet of a hooded seal pup during the first month consists almost exclusively of crustaceans (Haug et al. 2000). Later, for older seals, when associated with the pack-ice, the diet consists of prey species found at greater depths (Haug et al. 2004, 2007), with no apparent differences in prey composition between young (<2 yr) and older seals (Haug et al. 2007). Analysis of fatty acids in adipose tissue indicated, however, that juvenile (1 to 5 yr) and adult (>6 yr) hooded seals from the NWS differed significantly in their fatty acid profiles (Tucker et al. 2009), indicating a shift in diet as the seals mature. *B. pinnipedialis* has not been isolated from any of the cold-blooded hooded seal prey species, although cold-blooded species might act as a reservoir for *Brucella*-infection; *B. melitensis* biovar 3 was cultured from Nile catfish *Clarias gariepinus* (El-Tras et al. 2010), and *Brucella* spp. have been isolated from frogs (Eisenberg et al. 2012, Fischer et al. 2012). Exposure to *B. pinnipedialis* through the juvenile diet may explain the increased probability of seropositivity from pups to yearlings, as well as the subsequent decrease in seropositivity with increasing age and shift in prey species. *B. pinnipedialis* has also been isolated from pinniped lungworms (Garner et al. 1997), and pinnipeds are infected with these by consumption of fish (Measures 2001). *Brucella* spp.–specific DNA has also been detected in seabirds from the Northwest Atlantic (Bogomolni et al. 2008), suggesting that the marine environment may act as a reservoir for *Brucella* spp.

CONCLUSIONS

Our study suggests that *Brucella pinnipedialis* infection is not sustained in adult hooded seals. Since *Brucella* spp. grow poorly in the environment and each bacterial species infects a preferential host, it has been proposed that each species represents an evolutionary line adapted to a particular host and that the species in the genus *Brucella* diverged at the

same time as their hosts (Bourg et al. 2007). Recent work, however, using genetic mapping indicated that most *Brucella* species diverged around 86 000 to 296 000 yr ago (Foster et al. 2009). The point of divergence of the *B. pinnipedialis* is thus incompatible with the divergence of their hosts, since early pinnipeds originated 34 to 24 million yr ago and the split between Phocidae and Otarioidea occurred 12 million yr later (Higdon et al. 2007). An exposure of pinnipeds to *B. pinnipedialis* at a later point of time may hence better explain the transmission of *B. pinnipedialis* to pinnipeds. Our findings suggest an environmental source of infection for the hooded seal, rather than a horizontal or vertical transmission within the species. This is in line with the discoveries of *Brucella* species within a broader range of host species (Scholz et al. 2008, El-Tras et al. 2010, Eisenberg et al. 2012, Fischer et al. 2012) and exposure via heretofore unexpected pathways.

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