Prevalence of antibodies against *Brucella* spp. in West Greenland polar bears (*Ursus maritimus*) and East Greenland muskoxen (*Ovibos moschatus*)

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Abstract

Zoonotic infections transmitted from marine mammals to humans in European Arctic are of unknown significance, despite considerable potential for transmission due to local hunt and a rapidly changing environment. As an example, brucellosis may have significant impact on human health due to consumption of raw meat or otherwise contact with tissues and fluids of infected game species such as muskoxen and polar bears. Here we present serological results for Baffin Bay polar bears (*Ursus maritimus*) (*n* = 96) and North East Greenland muskoxen (*Ovibos moschatus*) (*n* = 32) for antibodies against *Brucella* spp. The analysis was a two-step trial initially using the Rose Bengal Test (RBT), followed by confirmative competitive enzyme-linked immunosorbent assays of RBT-positive samples. No muskoxen had antibodies against *Brucella* spp, while antibodies were detected in six polar bears (6.25%) rendering a seroprevalence in line with previous findings in other Arctic regions. Seropositivity was not related to sex, age or biometrics i.e. size and body condition. Whether the detected polar bear *Brucella* spp. antibodies found in polar bears were due to either prey spill over or true recurrent *Brucella* spp. infections is unknown. Our results therefore highlight the importance of further research into the zoonotic aspects of *Brucella* spp. infections, and the impact on wildlife and human health in the Arctic region.

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

Introduction

The Arctic ecosystem is subject to several interacting anthropogenic stressors that cause cumulative stress in humans and wildlife, which may in turn lead to increased susceptibility to zoonotic infections (Atwood et al. 2017; Jenssen et al. 2015; Greer et al. 2008; Hueffer et al. 2011; Sonne 2010). In some human populations in the Arctic, it is common to consume raw or insufficiently heat-treated wildlife and game meat (Tryland et al. 2013). The importance of heat-treatment is exemplified by studies of toxoplasmosis in North America, where 80% of examined humans were seropositive in an Inuit community with dietary preference for raw meat, as opposed to 10% seropositivity within a local Cree population having dietary preference for cooked foods (Lévesque et al 2007; Messier et al. 2009). Marine mammals including polar bears, are an important food source for people in the Arctic, yet the burden of zoonotic pathogens in these species remains largely unknown in most Arctic regions. While human cases of trichinosis and digital mycoplasmosis ("seal-finger") are typically reported (Rodahl 1952; Tryland et al. 2013), the pathogen-spectrum has rarely been addressed by systematic studies. In addition to marine mammals, muskoxen are also an important food resource in some parts of the Arctic. For example, in Greenland alone more than 2,000 muskoxen and 150 polar bears are harvested annually (Piniarneq 2016). In addition to dietary exposure, Arctic hunters are in frequent physical contact with raw tissues and fluids of hunted wildlife, most often lacking any preventive measures against transmission of zoonotic pathogens. Information about the occurrence of wildlife transmitted zoonotic diseases in the Arctic parts of Europe is generally limited (Jenkins et al. 2013; Tryland et al. 2013), while it has been studied more intensively in Arctic Canada (Campagna et al. 2011; Goyette et al. 2014; Lévesque et al. 2007; Messier et al. 2012; Sampasa-Kanyinga et al. 2013).

Brucella spp. are zoonotic Gram-negative coccobacilli causing the disease brucellosis in humans and animals such as domestic ruminants, pigs, and dogs (Fraser 1991; Godfroid et al. 2011; Metcalf et al. 1994) and in Arctic mammals including polar bears (*Ursus maritimus*) and muskoxen

(*Ovibos moschatus*) (Atwood et al. 2017; Godfroid 2002; Godfroid et al. 2011; Nymo et al. 2011). Although brucellosis is rarely fatal, depending on the *Brucella* spp. and host, it may cause a range of pathological processes such as mastitis, abortion, orchitis, and osteomyelitis (Davis 1990; Enright 1990; Ross et al. 1994; Brew et al. 1999; Prenger-Berninghoff et al. 2008; Siebert et al. 2009, 2017). Specific species of *Brucella* are rarely reported for marine mammals since there exist no specific or validated serological tests (Godfroid 2002). Culture or DNA isolation and sequencing can overcome problems of cross-reactivity, but such samples are rarely available in relation to wildlife sample collection. The wide spread zoonotic *B. suis* biovar 4, also called “rangiferine brucellosis”, has however been reported in muskoxen previously (Gates et al. 1984; Tomaselli et al. 2016).

As information regarding brucellosis in wildlife and the associated zoonotic risks are generally sparse for Greenland, the present study aimed at determining the seroprevalence of *Brucella* spp. exposure in West Greenland polar bears (*U. maritimus*) and East Greenland muskoxen (*O. moschatus*) to have a first assessment of the risk associated with handling, storage and consumption of these species.

Materials and methods

Sampling of polar bears

The sampling locality of polar bears from the West Greenland Baffin Bay subpopulation is shown in Figure 1. Serum samples (n = 96; Table 1) were obtained during a 5 years period (2009-2013) between Savissivik (ca. 76˚20´ N) and Uummannaq (ca. 70˚14´ N) (Laidre et al. 2012; SWG 2016). Polar bears were immobilised and handled according to standard procedures using 5-10 m Zoletil ® (200 mg/ml i.m.) from helicopter as described by Stirling et al. (1989). During immobilisation, blood samples were drawn from the femoral vein and a vestigial premolar (pm1) tooth was extracted for determination of individual age from analysis of incremental layers in the cementum. Blood samples were taken in plain vacutainers and following clotting, the blood was centrifuged at 1100g for 5 min.

The serum was pipetted off and transferred to cryovials, immediately frozen and stored at –20°C until analysis. Standard body measurements (standard length and axillary girth in cm) were taken and total body mass was estimated using the approach by Derocher and Wiig (2002). In the field, general body condition of individual polar bears was visually estimated on a scale from 1 to 5 according to Stirling et al. (2008), where 1 and 5 represent the leanest and most obese bears, respectively. According to this scale, polar bears in categories 3 and 4 are in "good condition". The individual age estimations were carried out by counting the cementum growth layer groups (GLGs) of the lower right rudimental premolar after decalcification, sectioning (14 µm) and staining with toluidine blue as described by Dietz et al. (1991). Polar bears were categorized as: cub of the year (COY), yearlings, two-year-old cubs, sub-adults and adults. Adult males were those ≥6 years of age, and adult females were ≥5 years of age according to Rosing-Asvid et al. (2002).

Sampling of muskoxen

Figure 1 shows the sampling locality of muskoxen. Serum samples from muskoxen (n = 32; Table 2) were obtained during two surveys for the study of muskox spatial ecology in North East Greenland, Zackenberg Valley, in 2013 and 2015. The muskoxen were immobilised and handled according to standard procedures described in Mosbacher et al. (2016) and Schmidt et al. (2016). Briefly, muskoxen were immobilized from the ground using a combination of etorphine, xylazine, medetomidine, and ketamine. Doses were for a 200 kg female muskox were: 2 mg (0.01 mg/kg i.m.) etorphine (Captivon 9.8 mg/ml; Wildlife Pharmaceuticals, White River, South Africa), 30 mg (0.15 mg/kg) xylazine (Rompun dry substance 500 mg; Bayer Animal Health, Denmark), 0.3 mg (0.0015 mg/kg) medetomidine (Zalopine 30 mg/ml; Orion Pharma Animal HealthDenmark) and 40 mg (0.2 mg/kg) ketamine (Ketaminol 100 mg/ml; MSD Animal Health, Denmark). Doses were supplemented with sterile water for injection and absolute ethanol to prevent freezing. Resultant total volumes were 1.5 ml and a concentration of 20 % ethanol. Blood samples were taken from the jugular vein in plain vacutainers and following clotting, the blood was centrifuged at 1100g for 5 min after which the serum was pipetted off and transferred to cryovials that were immediately frozen and stored at –20°C until analysis. The body condition score for muskoxen was determined by estimating the amount of soft tissue on rump, thorax and withers by palpation (Gerhart et al. 1996). Muskox age determination was based on horn development according to Olesen and Thing (1993). Only adult muskox individuals (aged 4 years of age or more) were handled and sampled.

Serological analyses

No specific or validated serological tests for *Brucella* infection in marine mammals have been developed and the detection of specific antibodies is based on tests used in terrestrial mammals (Godfroid 2002; Sonne et al. 2018). In an attempt to avoid problems of cross-reactivity and false-positives, two serological tests: the Rose Bengal Test (RBT) and the competitive-enzyme linked immuno-sorbent assay (C-ELISA), were performed to identify *Brucella* spp. antibodies in serum. According to the OIE Terrestrial Manual, the C-ELISA can eliminate some but not all false positive reactions due to cross-reacting bacteria such as Yersinia enterocolitica O:9 . According to the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Eloit and Schmitt 2017), the RBT is recommended as a general purpose diagnostic test in all wildlife species while the C-ELISA appear to be useful for seroepidemiological surveys in wildlife (Stack et al. 1999).

All samples were initially screened with a commercial RBT (PrioCHECK Brucella Rose Bengal Test, Prionics AG, Zürich, Switzerland), according to the manufacturer's instructions. In brief, one drop of test serum (30 µl), and one drop of Rose Bengal antigen were transferred to the test circle on the slide and mixed thoroughly. The slide was rotated for 4 minutes whilst examined for agglutination. A positive and negative control were used in each test run. Positive samples were confirmed with C-ELISA (SVANOVA Biotech AB, Uppsala, Sweden) according to the manufacturer's instructions. In brief, 45 µl of sample dilution buffer was added into each well used for serum samples, serum controls and conjugate controls, and 5 µl of positive, weak positive, and negative serum controls were added into appropriate wells. All control sera were run in duplicates. Five microliters of test sample were added in duplicates to the wells, and 50 µl of mAb-Solution were added to all wells used for controls and samples. The plates were incubated in 37ºC for 30 minutes. After incubation the plate was rinsed with buffer, and 100 µl Conjugate Solution were added into each well, followed by a second incubation at room temperature for 30 minutes. The plate was rinsed, and 100 µl Substrate Solution were added to each well and incubated for 10 minutes at room temperature before adding 50 µl Stop Solution to each well.

Optical density (OD) was assessed at 450 nm using a microplate photometer (air as blank) and the percent (%) 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%). A sample was regarded as seropositive to Brucella when it tested positive in both RBT and C-ELISA.

Results

None of the muskoxen tested positive for *Brucella* spp. antibodies by the RBT, and were thus not analysed in the C-ELISA. Of the polar bears, 7 animals (7.3 %) tested positive in the RBT, while the C-ELISA confirmed that 6 (6.3%) of the polar bears were true seropositive (Figure 2). The six polar bears with antibodies against *Brucella* spp. included one adult male sampled in 2010, two adult females sampled in 2010 and 2012, two sub-adults sampled in 2011 (male) and 2012 (female) and one yearling (male) sampled in 2010. *Brucella* spp. positive sero-status thus appeared equally distributed among adults and younger polar bears in our cohort.

Discussion

Our findings are comparable with data for these species from other Arctic regions (Tryland et al. 2001; Rah et al. 2005; O'Hara et al. 2010; Godfroid, 2012). Tryland et al. (2001) found a seroprevalence of 5.4% for *Brucella* spp. in 297 polar bears from Svalbard and the Barents Sea collected from 1990-1998, while a seroprevalence ranging from 5-17% was found in polar bears from Alaska (*n* = 500) and Canada (*n* = 275) collected between 2003 and 2006 and from 1982 to 1999, respectively (O'Hara et al. 2010; Rah et al. 2005). As in our study, the serological screenings of polar bears from Alaska did not shown any relationship between serostatus, sex and age of the bears (Rah et al. 2005). In contrast to this, the study on polar bears from Beaufort Sea revealed a higher seroprevalence in females than males (17 vs. 11%) and showed to be highest in animals aged 1-5 years (14%; *n* = 96; Rah et al. 2005).

The (sub)species of *Brucella* spp. bacteria involved and the source of infection in polar bears have been disputed (Godfroid 2012). Indirect measures of brucellosis such as antibody tests, are in general best supported by the isolation of *Brucella* spp., by which culture or genetic sequencing renders a valid suggestion of taxonomic subcategorization. However, samples other than blood were not available in the present study. Cross-reactivity in serologic assays between *Brucella* spp. and *Yersinia enterocolitica* is well-documented (Ahvonen et al. 1969; Corbel and Dag 1973; Bundle et al. 1984). However, in a study of seals and whales, both being polar bear prey, no cross reactivity between *Brucella* spp. and *Y. enterocolitica* was found (Tryland et al. (1999). These data strongly suggest that any observed antibody titres in muskoxen and polar bears of the present study were due to *Brucella* spp. infection.

It is a general assumption that brucellosis is transmitted to polar bears through ingestion of infected seals, whale or muskoxen (Tryland et al. 2001). In Alaska, *Brucella* spp. found in polar bears were found likely to be of terrestrial origin (O'Hara et al. (2010). Altogether, this suggest that the detected polar bear *Brucella* spp. antibodies found in the present investigation were due to either prey spill over or true *Brucella* spp. infections (Fraser 1991; Tryland et al. 2001). Further studies are therefore needed to address if *Brucella* spp. infections circulates among Greenland polar bears and whether it is associated with any pathology. Such investigations would allow a better prediction of *Brucella* spp. exposure and its significance for the health of North West Greenland polar bears.

Evidence of brucellosis in muskoxen is sparse. In consistency with our findings, an analysis of 132 muskoxen from North East Greenland in 1982 to 1983 revealed a seroprevalence for *Brucella* spp. of 0% (Clausen and Hjort 1986). On the other hand, Nymo et al. (2016) found recurring *Brucella* spp. antibody titres over time when analysing 52 muskoxen from Alaska (1982-2010). The seropositive muskoxen were from a part of Alaska with a high prevalence of *Brucella* spp. seropositive caribou (Zarnke et al. 2006). However, the North East Greenland muskox population is geographically isolated, and thus no spill over from other Arctic ungulate populations is likely to take place.

Serological screenings conducted in the North Atlantic and Greenland Sea indicate that brucellosis has a wide geographical distribution among marine mammals including e.g. seal spp. (Nielsen et al. 1996; Prenger-Berninghoff et al. 2008; Tryland et al. 1999, 2005). Greenland, with its subsistence hunters and marine predator interactions (e.g. polar bears and seals), comprises a unique opportunity to study the occurrence of zoonotic diseases in a One Health perspective while tying together human and ecological and wildlife health. Brucellosis is in general a major public health concern worldwide (Ross et al. 1996; Tryland et al. 2013). The presence of antibodies against *Brucella* spp. in polar bears shows that these predators are exposed to the bacterium, although the prevalence seems low (6.3%), but not if it is true infections or spill over from prey exposure. Only in the case of true infections present a significant zoonotic potential for those who are handling or hunted polar bears and consuming their meat. There was however no evidence of *Brucella* spp. exposure in East Greenland muskoxen, which indicates that they are likely not affected by *Brucella* spp. infections and thereby not presenting a risk in terms of being a source of zoonotic *Brucella* infection for handlers and hunters.

Conclusions

Since all 32 analysed muskoxen were seronegative, the East Greenland population of the species seems to be free from brucellosis. 6.3% of the 96 polar bears analysed were seropositive either due to prey spill over or due to recurrent *Brucella* spp. infections. There was no clear association between seropositivity and age or biometric parameters i.e. size and body condition of polar bears. We suggest further studies on the distribution and taxonomic characterisation of *Brucella* spp. in Greenland, to better understand their potential harmful effects on wildlife populations as well as their zoonotic potential.

Compliance with Ethical Standards

According to national legislation for studies of polar bears all polar bear samples were collected with permission of the Government of Greenland´s Department of Fishery, Hunting and Agriculture (Nuuk). File number 66.24/06: 11 February 2009, 24 February 2010, 24 March 2011 (2011 and 2012), and 25 March 2013. Capture and handling of muskoxen in this study followed the guidelines of the American Society of Mammalogists (Sikes et al. 2011), and research permits were granted by the Greenlandic government (j.no. G13-029 and G15-019) and by the Greenlandic police (j. no 55se-50190-00153-15). No conflict of interest were reported.

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TABLES

Table 1. Year and biometrics (weight, body condition and standard length) for the 96 West Greenland polar bears immobilised and serum sampled during 2009-2013. COYs: cub of the year, F: females, M: males. Weight: estimate body weight based on Derocher and Wiig (2002). Condition: body condition (1-5). SL: Standard length. Blanks: age/sex groups not immobilised and sampled.

	2009		**2010**		**2011**		**2012**		**2013**	
	Mean±SD (n)	Min-Max (n)	Mean±SD (n)	Min-Max (n)	Mean±SD (n)	Min-Max (n)	Mean±SD (n)	Min-Max (n)	Mean±SD (n)	Min-Max (n)
COYs F										
Weight (kg)					21.65 (1)	21.65 (1)	18.00 (1)	18.00 (1)		
Condition (1-5)					3 (1)	3 (1)	2 (1)	2 (1)		
SL (cm)					93 (1)	93 (1)	89.5 ± 3.54 (2)	87-92 (2)		
COYs M										
Weight (kg)							17.09±5.12 (2)	13.5-20.7 (2)		
Condition (1-5)							3 (2)	3-3 (2)		
SL (cm)							87±5.66 (2)	83-91 (2)		
Yearlings F										
Weight (kg)			72.6±14.9 (2)	62-83.2 (2)	85±13.4 (2)	75.5-94.5 (2)	108.6±10.8 (2)	100.9-116.2 (2)	57.8 (1)	57.8 (1)
Condition (1-5)			3 (2)	3-3 (2)	3 (2)	3-3 (2)	3 (3)	3-3 (2)	3 (1)	3 (1)
SL (cm)			140.5±10.6 (2)	133-148 (2)	155.5±3.54 (2)	153-158 (2)	159±2.8 (2)	157-161 (2)	134 (1)	134 (1)
Yearlings M										
Weight (kg)			104.5±21.9 (2)	89-120 (2)	117.9±17.9 (2)	105.1-130.5 (2)				
Condition (1-5)			3 (2)	3-3 (2)	3 (2)	3-3 (2)				
SL (cm)			154±9.9 (2)	147-161 (2)	167.5±4.9 (2)	164-171 (2)				
Two-year-old F										
Weight (kg)	131.2±29.6 (2)	110.3-152.1 (2)	160.7 (1)	160.7 (1)	115.9 (1)	115.9 (1)				
Condition (1-5)	3 (2)	3-3 (2)	3 (1)	3 (1)	3 (1)	3 (1)				
SL (cm)	169.5±12.0 (2)	161-178 (2)	179.0 (1)	179.0 (1)	167.0 (1)	167.0 (1)				
Two-year-old M										
Weight (kg)	149.2 (1)	149.2 (1)			182.6 (1)	182.6 (1)	136.3±43.4 (2)	105.6-167.0 (2)		
Condition (1-5)	3 (1)	3 (1)			3 (2)	3-3 (2)	3 (2)	3 (2)		
SL (cm)	184 (1)	184 (1)			182 (1)	182 (1)	169±15.6 (2)	158-180 (2)		
Subadults F										
Age (years)	4 (1)	4 (1)	3 (1)	3 (1)	2.5±0.71 (2)	2-3 (2)	3 (2)	3-3 (2)		
Weight (kg)	132.7 (1)	132.7 (1)	147.5 (1)	147.5 (1)	131±11.3 (2)	123-139 (2)	191.2±46.9 (2)	158-224.4 (2)		
Condition (1-5)	3 (1)	3 (1)	3 (1)	3 (1)	2.5±0.71 (2)	2-3 (2)	2 (2)	2-2 (2)		
SL (cm)	182 (1)	182 (1)	174 (1)	174 (1)	174.5±6.36 (2)	170-179 (2)	188±24 (2)	171-205 (2)		
Subadults M										
Age (years)	4 (1)	4 (1)	3.25±1.26 (4)	2-5 (4)	4±1 (3)	3-5 (3)	5 (1)	5 (1)		

Weight (kg)	214.0 (1)	214.0 (1)	192.1±32.1 (4)	161.7-234.1 (4)	232.9±12.7 (3)	225-247.6 (3)	283.2 (1)	283.2 (1)		
Condition (1-5)	3 (1)	3 (1)	2.5±0.58 (4)	2-3 (4)	3±1 (3)	2-4 (3)	2 (1)	2 (1)		
SL (cm)	198 (1)	198 (1)	192.5±8.96 (4)	184-205 (4)	208±12.49 (3)	194-218 (3)	222 (1)	222 (1)		
Adult F										
Age (years)	9.6±5.13 (5)	6-17 (5)	13.25±3.73 (8)	5-16 (8)	9.7±3.9 (11)	5-15 (11)	7.44±2.46 (9)	5-12 (9)	9 (1)	9 (1)
Weight (kg)	194.9±19.0 (5)	170.1-221.6 (5)	229.2±30.4 (8)	176.6-260 (8)	208±15.8 (11)	172.8-227.9 (11)	201.4±27.1 (9)	150.4-232.6 (9)	221.2 (1)	221.2 (1)
Condition (1-5)	2.4±0.55 (5)	2-3 (5)	2.63±0.52 (8)	2-3 (8)	2.8±0.6 (11)	2-4 (11)	2.55±0.53 (9)	2-3 (9)	2 (1)	2 (1)
SL (cm)	202.8±2.39 (5)	199-205 (5)	198.8±4.8 (8)	194-207 (8)	198.3±5.62 (11)	188-207 (11)	196.7±6.34 (9)	184-203 (9)	205 (1)	205 (1)
Adult M										
Age (years)	11.4±6.6 (5)	6-20 (5)	15.7±7 (3)	9-23 (3)	11.7±5.7 (7)	6-24 (7)	13.2±3.56 (5)	9-17 (5)	9 (1)	9 (1)
Weight (kg)	379.0±66.3 (5)	283.8-439.0 (5)	358.1±74.6 (3)	276.2-422.1 (3)	382.6±61.3 (7)	270.7-438.8 (7)	409.6±28 (5)	378.4-451.5 (5)	331 (1)	331 (1)
Condition (1-5)	2.8±1.1 (5)	1-4 (5)	2.33±0.58 (3)	2-3 (3)	2.57±0.53 (7)	2-3 (7)	3.4±0.55 (5)	3-4 (5)	3 (1)	3 (1)
SL (cm)	237.6±8.88 (5)	229-250 (5)	233.7±13.8 (3)	218-244 (3)	235.7±6.82 (7)	228-248 (7)	236±11.8 (5)	221-248 (5)	217 (1)	217 (1)

Table 2. Biological information of the 32 East Greenland muskoxen immobilised and serum sampled in 2013 and 2015. Males were not immobilised and sampled in 2015. F: females, M: males

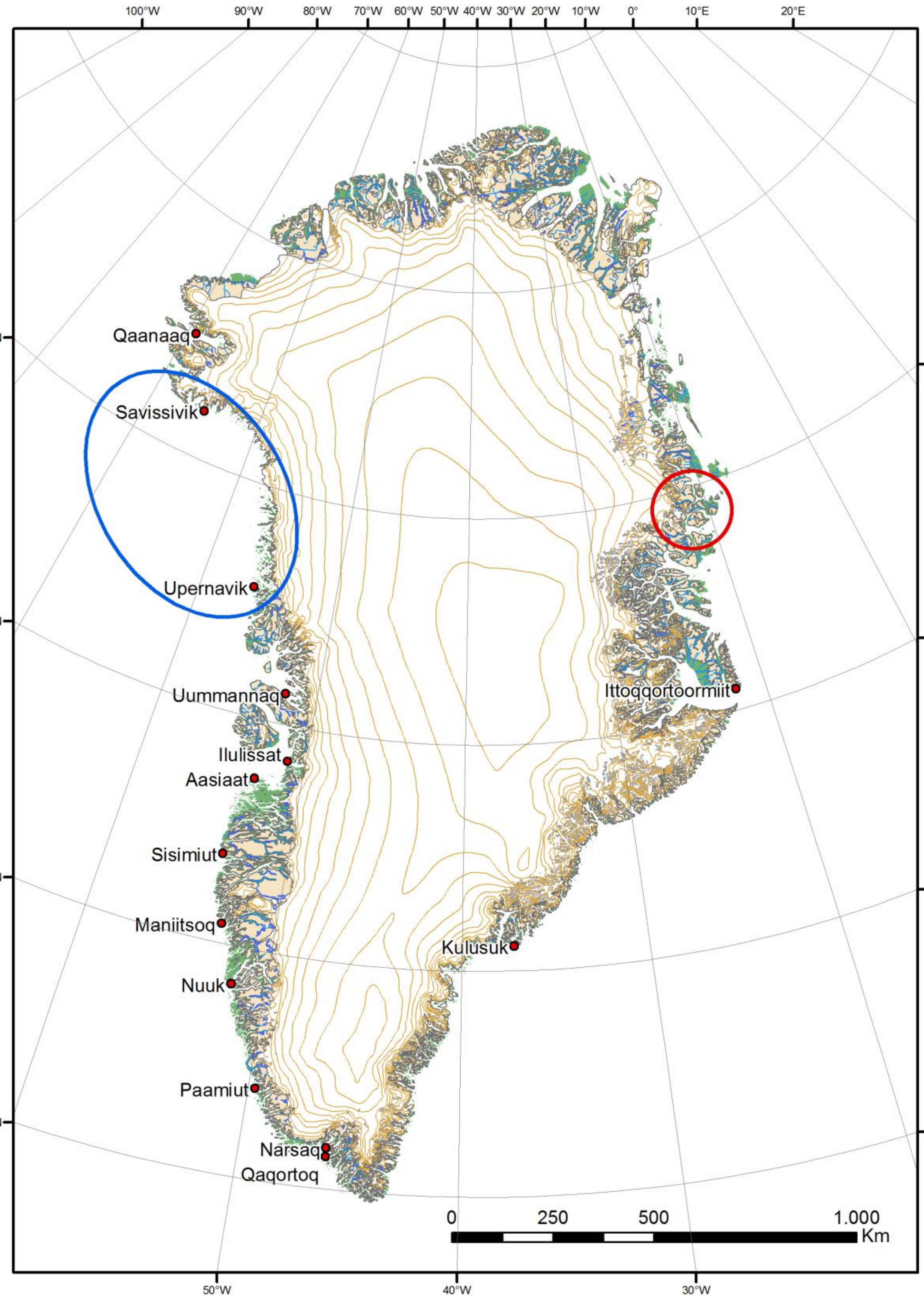
		2013		**2015**	
		Mean±SD (n)	Min-Max	Mean±SD (n)	Min-Max
Adult F	Weight	188.5±16.7 (13)	146-209	197.5±12.2 (14)	171.3-211.3
	Condition	4±0	4-4	4±0	4-4
Adult M	Weight	268±18 (5)	246-292	-	
	Condition	4±0	4-4		

FIGURE LEGENDS

Figure 1. Map showing the sample sites, numbers and years for North West Greenland polar bears and North East Greenland muskoxen included in the present study.

Figure 2. Seroprevalence for *Brucella* spp. among 96 North West Greenland polar bears sampled 2009-2013 based on RBT (n = 96) and subsequently confirmed by C-ELISA analyses (n = 6).

456 **FIGURES**



458 **FIGURE 1**

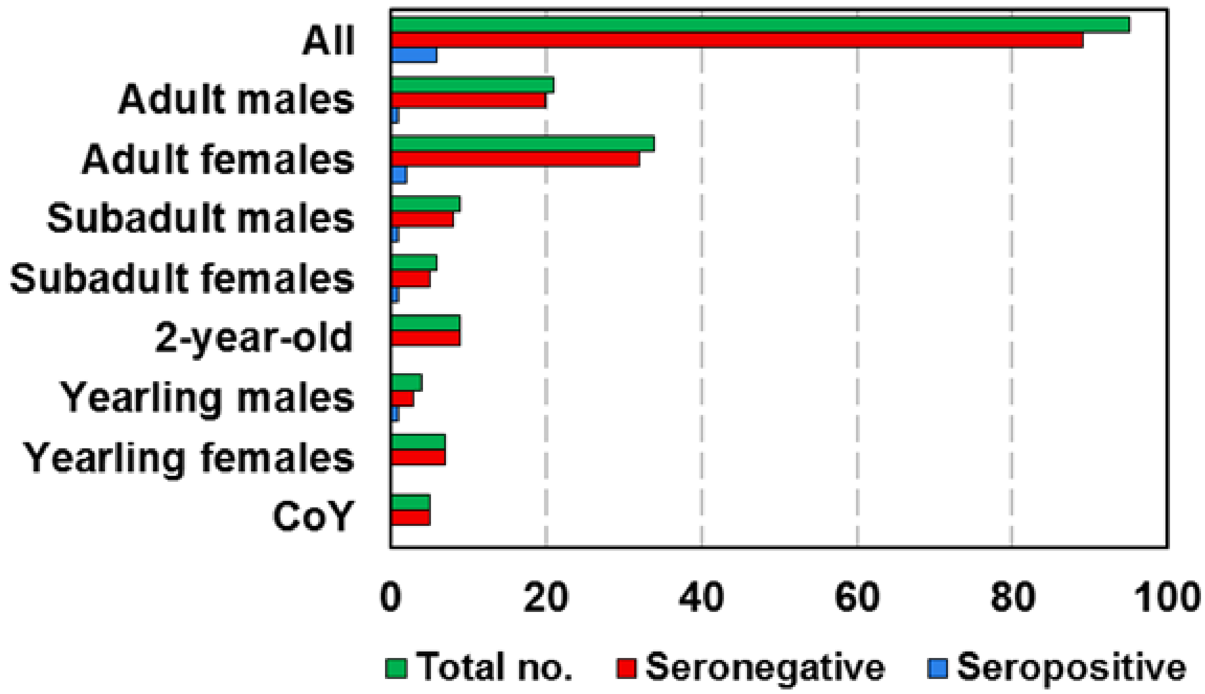

All
Adult males
Adult females
Subadult males
Subadult females
2-year-old
Yearling males
Yearling females
CoY
0
20
40
60
80
100
Total no.
Seronegative
Seropositive

FIGURE 2