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1 First isolation of *Brucella pinnipedialis* and detection of *Brucella* antibodies from
2 bearded seals (*Erignathus barbatus*)

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24 ABSTRACT: *Brucella* species infecting marine mammals was first reported in 1994
25 and in the years since has been documented in various species of pinnipeds and
26 cetaceans. While these reports have included species that inhabit Arctic waters, the
27 few available studies on bearded seals (*Erignathus barbatus*) have failed to detect
28 *Brucella* infection to date. We report the first isolation of *Brucella pinnipedialis* from a
29 bearded seal. The isolate was recovered from the mesenteric lymph node of a
30 bearded seal that stranded in Scotland and typed as ST24, a sequence type
31 associated typically with pinnipeds. Furthermore, serological studies of free-ranging
32 bearded seals in their native waters detected antibodies to *Brucella* in seals from
33 Chukchi Sea (1990-2011; 19 %) and Svalbard (1995-2007; 8 %), whereas no
34 antibodies were detected in bearded seals from the Bering Sea, Bering Strait or from
35 captive bearded seals.

36 KEY WORDS: Antibodies · bearded seal · *Brucella pinnipedialis* · isolation · MLST

37

38

INTRODUCTION

39 The isolation of *Brucella* from marine mammals was first reported in 1994 from four
40 free-ranging harbour seals (*Phoca vitulina*), two harbour porpoises (*Phocoena*
41 *phocoena*) and a common dolphin (*Delphinus delphis*), all inhabiting Scottish coastal
42 waters (Ross et al., 1994) and from an aborted foetus born to a captive bottlenose
43 dolphin (*Tursiops truncatus*) in the USA (Ewalt et al., 1994). Since these initial
44 reports, *Brucella* infection has become recognised in cetaceans and pinnipeds
45 inhabiting many of the world's oceans (Foster et al., 2002; Nymo et al., 2011) and
46 two species, *Brucella ceti* and *Brucella pinnipedialis*, have been described for
47 isolates with cetaceans and seals as preferred hosts, respectively (Foster et al.,

48 2007). These species are genetically distinct from *Brucella* associated with terrestrial
49 mammals (Whatmore et al., 2016).

50 With respect to Scottish coastal waters, *B. pinnipedialis* has been recovered from the
51 other resident species, grey seals (*Halichoerus grypus*) as well as from hooded seals
52 (*Cystophora cristata*), which are occasional visitors to the region (Foster et al., 1996;
53 2002). The isolation of *B. pinnipedialis* has also been reported from hooded seals in
54 their native Arctic waters and from harbour and grey seals elsewhere in Europe
55 (Nymo et al., 2011). Further afield, *B. pinnipedialis* has been cultured from other
56 pinniped species including Pacific harbour seal (*Phoca vitulina richardsi*) (Garner et
57 al., 1997), ringed seal (*Pusa hispida*), harp seal (*Pagophilus groenlandica*) (Forbes
58 et al., 2000) and California sea lion (*Zalophus californianus*) (Goldstein et al., 2009).
59 Serological studies provide further presumptive evidence that *Brucella* infections are
60 widespread amongst other pinniped species, including some resident in the
61 Southern Hemisphere (Nymo et al., 2011). Taken together, culture and serological
62 evidence (Foster et al., 2002; Nymo et al., 2011), indicate that *Brucella* is endemic in
63 many of the marine mammals that inhabit the world's open oceans and seas.
64 Seropositive animals, however, can be due to immunological cross-reactions, in
65 particular *Yersinia enterocolitica* serotype O9, however, this strain has not been
66 recovered from marine mammals to date (Ross et al., 1996 and pers. obs. GF) to an
67 organism from a different genus; thus the isolation of *Brucella* by cultural methods,
68 remains the gold standard of definitive proof of infection in different hosts and
69 discrete populations of marine mammals.

70 There have been few reports on studies of *Brucella* infection in bearded seals
71 (*Erignathus barbatus*) to date, but where performed, no evidence of exposure was

72 found (Calle et al., 2008; Tryland et al., 1999). Bearded seals are members of the
73 Phocidae family and represent the only species within the genus *Erignathus*. They
74 have a patchy circumpolar distribution throughout the Arctic and subArctic between
75 45 and 85° N. Two sub-species are recognised, *Erignathus barbatus barbatus*, which
76 ranges from the central Canadian Arctic eastwards to the central Eurasian Arctic and
77 *Erignathus barbatus nauticus*, which ranges from the central Canadian Arctic
78 westwards to the Laptev Sea, Russia. The availability of sea ice to breed, moult and
79 rest on, in shallow water areas, is thought to be an important factor governing the
80 distribution of this benthic-feeding seal (Burns, 1981; Kovacs, 2016). In a review of
81 their extralimital records, bearded seals have been reported from the Netherlands,
82 France and Spain in the Eastern Atlantic and the island of Rügen in the Baltic Sea
83 (van Bree, 2000). Sightings in the UK are rare, with most modern reports occurring
84 around the Scottish coast, including the Shetland and Orkney Islands and single
85 sightings from the Isle of Mull, Aberdeenshire and Fife (JNCC/Defra, 2013).

86 This paper documents the first recovery and characterisation of *B. pinnipedialis* from
87 a bearded seal. The results of a serological study of free-living bearded seals in
88 Arctic waters and captive members of the species kept at the aquarium 'Polaria' in
89 Tromsø, Norway are also presented.

90

91

MATERIALS AND METHODS

92

Bearded seal necropsy

93 In early February, 2012, a stranded bearded seal (M61/12) was reported to the
94 Scottish Marine Animal Strandings Scheme (SMASS). The juvenile male animal had

95 stranded dead at Annachie Lagoon, St Fergus on the Aberdeenshire coast of the
96 north-eastern Scottish mainland (57°34'10.74"N 001°49'22.02"W) and represented
97 the first report of a stranded bearded seal in Scotland since records began in 1992.
98 The carcass was transported to SAC Consulting Veterinary Services, Inverness for a
99 post mortem examination performed according to a standard protocol (Dierauf,
100 1994). Samples of brain, lung, liver, spleen, kidney, mesenteric lymph node, urinary
101 bladder and small intestine were cultured on Columbia sheep blood agar (CSBA)
102 (Oxoid, Basingstoke, UK) and Farrell's medium (FM) (Farrell, 1974), incubated at 37
103 °C in air with 5 % added CO₂ as described previously (Foster et al., 2002). Plates
104 were examined for growth, daily, for 4 days and at frequent intervals thereafter up to
105 14 days. Isolates with colonial appearance typical of *Brucella* were tested initially for
106 Gram reaction, cellular morphology, acid-fastness with the modified Ziehl-Neelsen
107 stain, agglutination with *Brucella abortus* antiserum (Remel, Basingstoke, UK) and
108 ability to grow in air without added CO₂. Further testing included urea hydrolysis, H₂S
109 production, inhibition by basic fuchsin at 1/50,000 and 1/100,000, agglutination with
110 monospecific antisera A and M and lysis by phages TB, Wb, BK2, Fi, Iz and R/C all
111 at Routine Testing Dose. Multilocus sequence typing (MLST) using a 9 locus
112 scheme was performed as described previously (Whatmore et al., 2007). Tissue
113 samples for histological examination (whole brain, trigeminal ganglion, skin, thyroid
114 gland, adrenal gland, urinary bladder, spleen, lung, kidney, heart, and pancreas)
115 were collected, trimmed and processed routinely through graded alcohols and
116 embedded in paraffin wax prior to sectioning (5µm), mounting on glass microscope
117 slides and staining with haematoxylin and eosin. Blood was collected from the left
118 ventricle of the heart using a sterile needle into heparinised and plain vacutainers 6

119 ml vacutainers (BD, Wokingham, UK) for serology and urine analysis was performed
120 using the Combur 9 Test (Roche, Burgess Hill, UK).

121 **Serology**

122 The Alaska Department of Fish and Game Ice Seal program recovered serum from
123 heart blood samples from subsistence harvested bearded seals. Seals were shot on
124 sea ice by Alaska native hunters, as allowed under the Marine Mammal Protection
125 Act of 1972, in the Chukchi and Bering Strait off the north and northwest coasts of
126 Alaska, during May, June, July and October. In addition, 17 seals were sampled
127 immediately post mortem during 1978-1979 scientific collections conducted April-
128 June by the Outer Continental Shelf Environmental Assessment Program during
129 United States National Oceanographic and Atmospheric Administration cruises in the
130 Bering Sea under National Marine Fisheries permit #194 (Figure 1). Samples from
131 bearded seals from Svalbard were obtained from both dead and live animals.
132 Sixteen animals were shot on the ice as part of Norwegian scientific catches to
133 address stocks and diets for different seal species and their role in the marine
134 ecosystems and interactions with fisheries (1992-1995). Blood was collected on the
135 ice when cutting the main blood vessels to the fore flippers during standard bleeding-
136 out procedures of seals (Tryland et al., 1999). From live bearded seals (pups; 1995-
137 2007) blood was obtained from the extradural intra-vertebral vein using an 80 mm
138 needle (14 gauge, 2.1 mm) mounted on a 50 ml syringe. Blood was transferred into
139 blood collecting tubes (Venoject, Terumo, Leuven, Belgium) and serum was
140 prepared (3000 g, 15 min) and stored at -20 °C until analysis. Sex and age category
141 (pup < 1 year, juvenile < 3 years, adult > 3 years) were known for some or all of the
142 seals at each location (Table 1). Furthermore, blood samples were obtained from 5

143 bearded seals kept in captivity at the aquarium “Polaria” in Tromsø. Blood was
144 drawn from the plantar venous plexus of the hind flippers, using a 0.8 x 50 mm
145 needle and blood collecting tubes (Venoject). Serum was prepared by centrifugation
146 at 3 000 g for 15 min and serum stored at -20 °C until analysis.

147 These animals, initially captured in the wild in Svalbard, had been kept in captivity
148 since they were approximately 5 weeks of age; the seals interact extensively with
149 humans through training and feeding (Stokke, 2010). They were 9-10 years of age at
150 the time of sampling and had been trained to tolerate handling and blood sampling
151 (Table 1).

152 Serum samples (n = 205) were analyzed for anti-*Brucella* antibodies with a Protein
153 A/G indirect enzyme-linked immunosorbent assay (ELISA) as described previously
154 (Nymo et al., 2013a). The mean optical density (OD) of duplicate wells was
155 expressed as a percentage of the reactivity of a seal positive control: $([OD$
156 $sample/OD\ positive\ control] \times 100) = \text{percent positivity (\%P)}$. The cut-off was 73.6
157 %P.

158 **Statistical Analysis**

159 All statistical analysis was performed in JMP 11 Pro (SAS Institute, Medmenham
160 Marlow, UK).

161

162

163

RESULTS

164

Bearded seal necropsy

165 The carcase of M61/12 was fresh and had been chilled, but not frozen, prior to
166 necropsy, two days after notification. The animal was 149 cm in total length, 79
167 cm girth behind the front flippers and in moderate to poor body condition with a
168 mid-sternal blubber thickness of 16 mm.

169 Hair loss ranged from complete over the ventral surface of the animal through partial
170 coverage over the flank with bilateral symmetrical zones of alopecia, back to almost
171 complete hairloss over the perilumbar region. The head and neck exhibited almost full
172 coverage, excepting significant alopecia periocularly and over dorsal muzzle. In addition,
173 the foreflippers exhibited partial alopecia over the carpal and phylangeal regions. No
174 regions showed evidence for hair regrowth.

175 The oesophagus and stomach contained a notable amount of sand, and marine
176 debris comprising a fragment of worn black plastic sheet 45 mm long and a single
177 round pebble 1 cm in diameter was recovered from the stomach. No prey items
178 were found. Thyroid glands were grossly unremarkable. The lungs and cerebral
179 vessels were markedly congested, the bladder mucosa was grossly reddened and
180 the urine was turbid and dark red in colour and a high level of haemoglobin (ca 50
181 erythrocytes per μL) was detected with the Combur 9 Test. The brain showed diffuse
182 dilation of cerebral vessels but the cerebrospinal fluid was unremarkable.

183 **Bacteriology**

184 Small numbers of colonies typical of *Brucella* were recovered from the mesenteric
185 lymph node on CSBA and FM after four days. In addition, *Vibrio alginolyticus* was
186 recovered from multiple tissues. Cells of suspect *Brucella* colonies were tiny Gram
187 negative cocco-bacilli, which were acid-fast when tested in the modified Ziehl-
188 Neelsen stain. Agglutination was obtained in slide tests with *B. abortus* antiserum.

189 The strain required CO₂ for growth, was urease positive, H₂S negative and A
190 dominant. Growth was inhibited by basic fuchsin at 1/50,000 and 1/100,000 and
191 cultures were completely lysed by Tb phage, partially lysed by Wb, BK2 and Iz, with
192 no lytic effect with Fi and R/C. The strain was identified by MLST as *Brucella*
193 *pinnipedialis* sequence type (ST) 24.

194 **Histopathology**

195 The most significant histological change in M61/12 consisted of moderate, multifocal
196 granulomatous and eosinophilic meningo-encephalitis within the brain, often centred
197 on degenerate or intact nematode parasite larvae, with perivascular cuffing and
198 multifocal haemorrhages. The nematode larvae were not identified but gross
199 morphology of worms seen in the stomach were consistent with Anasakid
200 nematodes, *Pseudoterranova bulbosa* or *Contracaecum osculatum*. It is plausible
201 that the granulomatous foci in the brain were the result of aberrant tissue migration
202 of L4 larval stages from these species. Mild, multifocal histiocytic and eosinophilic
203 pneumonia (likely parasitic) was also noted along with moderate splenic histiocytosis
204 with mild lymphodepletion. The skin lesions consisted of mild epidermal hyperplasia
205 with follicular atrophy with no evidence of vasculitis or dermal necrosis. Moderate to
206 marked thyroid follicular hyperplasia and moderate to marked bilateral adrenocortical
207 hyperplasia were present. The most significant lesions and likely cause of death,
208 were multiple granulomatous foci in many regions of the brain consistent with
209 migrating nematode larvae. Overall, the seal appeared to have indications of chronic
210 morbidity and malnutrition/pica which, given the extralimital nature of this case, could
211 be due to pathogen exposure and/or inadequate feeding capacity.

212 **Serology**

213 Antibodies to *Brucella* were detected in 22 of 200 (11 %) serum samples collected
214 from wild bearded seals in Alaska and Svalbard (Table 1). Sixteen of the
215 seropositive seals came from 86 (19 %) animals that were subsistence harvested in
216 the Chukchi Sea between 1990 and 2011; one juvenile female, two juvenile males,
217 four adult females, one adult male, five females with unknown age class, one male of
218 unknown age and two animals of unknown sex and age (Table 1). The other
219 seropositive bearded seals, 6 of 76 (8 %), were all captured in the Svalbard
220 archipelago during the period 1995 to 2007. The positive animals were three female
221 and two male pups and the mother of one of the female seropositive pups. It is not
222 known whether the mothers of the other seropositive pups were amongst the
223 animals sampled. Antibodies to *Brucella* were not detected from any of the 38
224 bearded seals subsistence harvested in the Bering Strait or collected in the Bering
225 Sea or from the five animals kept in captivity at "Polaria" (Table 1). *Brucella*
226 antibodies were detected in the blood collected from the necropsied animal
227 (M61/12).

228 The average %P of the seropositive seals was 87,7 %P (SD 9,4) and the average
229 %P of the seronegative seals was 25,9 %P (SD 21,6). The material included six
230 mother/pup pairs from Svalbard sampled between 06.05.95 and 25.05.95. One
231 mother/pup pair was seropositive (mother: 93,2 %P, pup: 88,8 %P). The remaining
232 pairs had %P values below the cut-off, however, the %P of the pup could be
233 predicted from the %P of the mother with the following formula: $\text{pup} = -75,8 +$
234 $1,7 * \text{Mother}$, $r^2 = 0,84$.

235 **DISCUSSION**

236 This study documents for the first time the recovery of *Brucella* from a bearded seal,
237 as well as the first serological evidence of *Brucella* exposure in this host. Antibodies
238 were detected in sera from two of the four groups of free-ranging bearded seals
239 sampled; the Chukchi Sea (19 %) and the Svalbard archipelago (8 %), however,
240 they were not detected from 38 bearded seals from the Bering Strait region or the
241 Bering Sea (Table 1). A previous small-scale study also failed to detect *Brucella*
242 antibodies from six bearded seals taken during a subsistence hunt at St Lawrence
243 Island in the Bering Sea (Calle et al., 2008), so evidence of exposure to *Brucella* in
244 this region remains lacking (Figure 1). The Pacific bearded seals are not distinct
245 populations, they move from the Bering Sea through the Bering Strait with the
246 advancing and retreating ice edges. The detection of seropositive bearded seals
247 from the Chukchi Sea therefore may be significant for *Erignathus barbatus nauticus*
248 across their entire area. Another serological study for *Brucella* in bearded seals did
249 not detect antibodies from two locations in the North Atlantic, while antibodies were
250 detected in the other three sympatric species sampled; hooded, harp and ringed
251 seals (Tryland et al., 1999).

252 Typing of the *Brucella* isolate by MLST (Whatmore et al., 2007) demonstrated that it
253 belonged to the ST24 lineage of *B. pinnipedialis*. Sequence type 24 is the less
254 common of two STs isolated predominantly from pinnipeds (Groussaud et al., 2007)
255 and has previously been found associated with harbour seals, grey seals and a
256 minke whale (*Balaenoptera acutorostrata*) which stranded in Scotland and from
257 harbour seals and a beluga whale (*Delphinapterus leucas*) from North America
258 (Groussaud et al., 2007; Whatmore et al., 2017).

259 *Brucella*-associated pathology was not found either grossly or histologically, although
260 histology was not performed on the lymph node and an association of *B.*
261 *pinnipedialis* with the death of this animal was not established. This is in line with
262 previous findings, which have revealed a paucity of pathologies following *Brucella*
263 isolation from pinnipeds, including several apparently healthy harbour seals which
264 had been shot by fishermen (Foster et al., 2002). In contrast, a broad range of
265 pathologies have been reported for *Brucella* infection of various cetacean species
266 which include lymphocytic meningoencephalitis, sub-cutaneous lesions, blubber
267 abscessation, liver abscess, hepatic and splenic necrosis, macrophage infiltration in
268 liver and spleen, lymph node inflammation, pneumonia, peritonitis, mastitis,
269 osteomyelitis, spinal discospondylitis, diseased atlanto-occipital joint, endocarditis,
270 epididymitis and abortion (Foster et al., 2002; Nymo et al., 2011; Guzman-Verri et
271 al., 2012).

272 *In vitro* work has revealed differences between the classical terrestrial *Brucella*
273 strains and *B. pinnipedialis*. The *B. pinnipedialis* reference strain NCTC 12890 and
274 *B. pinnipedialis* hooded seal strains were eliminated from murine and human
275 macrophage cell lines, and a human epithelial cell line within 72-96 h (Larsen et al.,
276 2013b). Even more rapid elimination patterns were observed in hooded seal primary
277 alveolar macrophages (Larsen et al., 2013a) and epithelial cells (Larsen et al., 2016).
278 *Brucella pinnipedialis* NCTC 12890 was also found to be attenuated in the BALB/c
279 *Brucella* mouse model (Nymo et al., 2016). The reduced virulence in these models,
280 when compared to the terrestrial virulent strain *Brucella suis* 1330 (Larsen et al.,
281 2013b; Nymo et al., 2016), is in line with the limited virulence of the *B. pinnipedialis*
282 strains in their natural hosts (Foster et al., 2002). Five seropositive pups were detected
283 in the present study, all from Svalbard. The sampling took place in May and peak birthing for

284 bearded seals at Svalbard is in early May. The pups are thereafter weaned in approximately
285 24 days (Kovacs, 2016). The seropositive pups were hence of very young age. At least one
286 of the seropositive pups in the present study was the pup of a seropositive mother and a
287 strong relationship was identified between the titres (i.e. antibody levels) in the mothers and
288 the pups in the six mother/pup pairs from Svalbard. These findings suggest a transfer of
289 maternal antibodies between mother and pup. Seals have an endotheliochorial placenta
290 (Stewart and Stewart, 2009) where solely 5-10 % of the maternal antibodies are transferred
291 to the fetus *in utero*. Passive immunity via the colostrum is therefore essential in species in
292 which the type of placentation impedes contact between maternal and foetal circulation
293 systems, hindering the transfer of antibodies (Tizard, 2000). Indeed, when evaluating total
294 IgG levels in harbour seal mother and pups the mothers showed a decreasing trend during
295 lactation, while the total IgG levels in the pups were low at birth and higher at the end of
296 lactation (Ross et al., 1993). In dogs, which also have an endotheliochorial placenta (Stewart
297 and Stewart, 2009), pups from *Brucella canis*-infected bitches have antibodies against *B.*
298 *canis* (Carmichael and Kenney, 1970). In animals having an epitheliochorial placenta the
299 young are born virtually agammaglobulinemic, however, after receiving colostrum calves
300 from dams with high and low *Brucella*-antibody levels had corresponding high and low
301 *Brucella*-antibody levels (Sutherland et al., 1990). Maternal transfer of antibodies against
302 phocine distemper virus has been shown in harbour seals (Garnier et al., 2014) and
303 maternal and pup antibody titres were shown to be strongly correlated in Scottish grey seals
304 (Pomeroy et al., 2005). Even though the number of mother/pup pairs investigated in the
305 present study is low, our findings and the literature supports that the *Brucella*-antibody levels
306 of the pups are likely a reflection of the *Brucella*-antibody levels of the mothers and the result
307 of maternal transfer of antibodies rather than a vertical infection of the pups. However,
308 further bacteriology work on organ samples from mother and pup pairs is needed in order to
309 draw any conclusions on this matter. For hooded seals, however, no relation was found
310 between *Brucella* serostatus and ovulation rate or neonatal body condition (Nymo et al.,
311 2013b).

312

313 Bearded seals are largely solitary animals (Kovacs, 2016). Ringed seals and hooded
314 seals, from which *B. pinnipedialis* has been isolated and anti-*Brucella* antibodies
315 detected (Forbes et al., 2000; Nymo et al., 2013b) are also generally described as
316 being largely solitary (Kovacs, 2002; Miyazaki, 2002), though all three of these
317 species do gather in the same areas where habitat is suitable for breeding, moulting
318 and foraging. Contrary to cetaceans where vertical transmission of *Brucella* was
319 suggested (Ohishi et al., 2016), no evidence of vertical transmission of *Brucella* in
320 true seals has been reported. Furthermore, the solitary behaviour of bearded seals
321 suggests that opportunities for *Brucella* transmission between conspecifics are
322 restricted. Altogether, this re-enforces the possibility that *Brucella* infection may be
323 acquired from the environment, possibly via diet, as suggested previously (Lambourn
324 et al., 2013; Nymo et al., 2013b). In contrast, harp seals have also been shown to
325 harbour infections with *B. pinnipedialis* (Forbes et al., 2000; Tryland et al., 1999) but
326 this species demonstrates a much stronger tendency to congregate (Lavigne, 2002)
327 and transmission between conspecifics cannot be excluded.

328 Brucellosis is a significant zoonotic infection, which causes a broad range of
329 manifestations, especially associated with farmed animals and their products,
330 infected with *Brucella melitensis*, *B. abortus* and *B. suis*, but also *Brucella canis*
331 contracted from dogs. Whilst, there have been three reports of human infections with
332 marine mammal *Brucella*, none have involved *B. pinnipedialis*. Human infection has
333 been reported in a laboratory infection scenario with ST23, a clade predominantly
334 associated with porpoises, while naturally occurring infections have been reported
335 only with ST27 (Whatmore et al., 2008), only isolated thus far from bottlenose

336 dolphins (*Tursiops truncatus*) and California sea lions in the USA (Whatmore et al.,
337 2017) and recently, from a single bottlenose dolphin in the Mediterranean (Cvetnik et
338 al., 2016).

339 While the lack of human infections with *B. pinnipedialis* are in contrast to the findings
340 with *B. ceti* and the classical *Brucella* spp. mentioned above, the zoonotic potential
341 of *B. pinnipedialis* remains unknown at present. It is advisable, therefore, that those
342 working with bearded seals and other pinniped species consider the infectious
343 nature of the genus and follow appropriate safety procedures (Dierauf and Gulland,
344 2001).

345 In conclusion, we report the first isolation of *Brucella pinnipedialis* from a stranded
346 extra-limital juvenile male bearded seal. In contrast to cetaceans, reports of *Brucella*-
347 associated pathology in pinnipeds is lacking. Our study also provides novel
348 serological evidence for *Brucella* spp. exposure in free-ranging bearded seal
349 subpopulations. Future serological surveys and the isolation and characterization of
350 *Brucella* isolates from stranded and free-ranging bearded seals, as well as other ice
351 seals (ringed seal; ribbon seal; spotted seal) are needed to better understand the
352 significance of *Brucella* infection in these northern pinnipeds

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