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SEROLOGIC SURVEY OF *BRUCELLA* SPP. ANTIBODIES IN SOME MARINE MAMMALS OF NORTH AMERICA

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ABSTRACT: A serologic survey of anti-*Brucella* spp. antibodies was undertaken on 2,470 samples of 14 North American marine mammal species collected between 1984–97. Serum or blood from eight species of cetaceans and six species of pinnipeds was sampled from Pacific, Atlantic, and Arctic oceans. Two competitive enzyme-linked immunosorbent assays (C-ELISA's), using specific monoclonal antibodies to *Brucella abortus* cell wall components, were used to detect anti-*Brucella* spp. antibodies in the samples. Sera from 33 cetaceans and 61 pinnipeds gave inhibition values, in one or both of the tests, which exceeded the threshold that indicates *Brucella* spp. exposure in cattle. Seropositive animals were identified from Pacific, Atlantic, and Arctic oceans. While *Brucella* spp. was not isolated, differences in the response of seropositive cetacean and pinniped sera in the two assays suggest that two antigenically distinct species or biovars of *Brucella* spp. are present. No pathology consistent with clinical brucellosis was noted in any of the animals tested although detailed examination was not conducted on all carcasses.

Key words: *Brucella* spp., brucellosis, competitive ELISA, enzootic infection, marine mammals, serology.

INTRODUCTION

Brucella spp. that differ from recognized species or biovars have recently been isolated from a variety of marine mammal species both in the USA and in the UK (Ewalt et al., 1994; Foster et al., 1996; Garner et al., 1997; Clavareau et al., 1998). While the pathological significance of these bacteria has not yet been established, one isolate was collected from an aborted bottlenose dolphin (*Tursiops truncatus*) fetus (Ewalt et al., 1994), and other isolates were collected from subcutaneous lesions found on beached carcasses from three dolphin species (Foster et al., 1996). Although little is known about the epidemiology of brucellosis in marine mammals, there is now serological evidence that *Brucella* is present in marine mammals in British waters (Ross et al., 1994; Jepson et al., 1997), the Canadian Arctic (Nielsen et al., 1996), and on the northwest coast of USA (Garner et al., 1997).

Given the potential of *Brucella* spp. to cause zoonotic infections, the finding of seropositive Atlantic walrus (*Odobenus rosmarus rosmarus*) and ringed seals (*Phoca hispida*) among animals harvested by aboriginal hunters in the eastern Canadian Arctic (Nielsen et al., 1996) is of concern. Here we expand on those studies and report the prevalence of *Brucella* spp. antibodies in six species of pinnipeds and in eight species of cetaceans from the Arctic, Atlantic, and Pacific Oceans of North America.

MATERIAL AND METHODS

Pinnipeds

Samples from ringed seals (n = 628), and walrus (n = 170) were collected by Inuit or Department of Fisheries and Oceans personnel from eight locations in Arctic Canada and Newfoundland between 1984 and 1997 (see Table 1 for map coordinates). Samples were collected as soon as possible after death and held at −20 C. Whole blood was usually collected and in a few cases serum was separated before freezing. Occasionally, it was necessary to obtain blood from partially thawed liver samples. Prior to testing, both blood and sera were centrifuged

TABLE 1. Serum antibody prevalences to *Brucella* spp. in seven species of pinnipeds from North America.

Location	Coordinates	Year	Species	Number	Positive[a]
US Atlantic Coast	45°00′N, 67°00′W to 40°30′N, 74°00′W 41°51′N, 70°00′W to 41°39′N, 70°42′W 40°59′N, 66°24′W	1987–93	Harbor seal[b]	8	4 (50.0)[a]
St. Lawrence Estuary	47°00′N, 71°00′W to 49°30′N, 67°20′W	1995–96	Harp seal	89	0
		1995–96	Gray seal	23	0
		1996	Hooded seal	4	1 (25.0)
		1995–96	Harbor seal	96	3 (3.1)
Gulf of St. Lawrence	46°00′N, 59°40′W to 49°30′N, 67°20′W	1988–93, 96, 97	Harp seal	269	5 (1.8)
		1994, 96, 97	Gray seal	129	4 (3.1)
		1989–93, 96, 97	Hooded seal	194	7 (3.6)
Amet Island, NS	45°50′N, 63°10′N	1991–94	Gray seal	67	2 (3.0)
Sable Island, NS	43°55′N, 60°00′W	1992, 94	Harbor seal	26	7 (26.9)
		1992, 94	Gray seal	36	4 (11.1)
		1994	Hooded seal	1	1 (100)
Newfoundland	50°00′N, 59°00′W to 53°00′N, 54°00′W	1995–97	Harp seal	95	3 (3.1)
		1997	Hooded seal	5	1 (20.0)
		1996–97	Ringed seal	18	1 (5.5)
Vancouver Island	49°00′N, 125°10′W to 49°00′N, 123°30′W	1992, 93	Harbor seal	33	7 (21.7)
Nunavik	56°32′N, 76°33′W to 58°41′N, 65°57′W	1996	Ringed seal	27	0
		1995	Walrus	4	0
Arviat	61°07′N, 94°04′W	1990–92	Ringed seal	275	0
Pangnirtung	66°09′N, 65°43′W	1990, 92, 93, 95, 96	Ringed seal	208	6 (2.9)
Grise Fjord	76°25′N, 82°54′W	1996	Walrus	5	0
		1992	Ringed seal	67	0
		1993	Ringed seal	14	0
Resolute Bay	74°42′N, 94°50′W	1996	Walrus	4	0
Hall Beach	68°46′N, 81°13′W	1988, 92, 96	Walrus	67	3 (4.5)
Igloolik	69°23′N, 81°48′W	1984, 87, 88, 92, 93, 96	Walrus	90	2 (2.2)
Grand total				1,855	57 (3.1)

[a] Number positive (percent positive).
[b] Stranded animals.

at 1000 × *g* for 10 min to remove cellular debris.

Ages of ringed seals were determined by counting dental annuli in transverse sections of canine teeth (Smith, 1973). Females < 5-yr-old and males < 7-yr-old were considered juveniles (MacLaren, 1958). Ages of walrus were determined by counting dental annuli in the teeth (Garlich-Miller et al., 1993). Females < 6-yr-old and males < 8-yr-old were considered to be juveniles (Fay, 1985).

Harbor seals (*Phoca vitulina*) were blood sampled during March, June, October, and December 1992 and 1993 on Vancouver Island (British Columbia, Canada; $n = 33$) 1992–94 on Sable Island (Nova Scotia, Canada; $n = 26$) and in 1995 and 1996 in the St. Lawrence Estuary $n = 96$ (Table 1). The capture method for harbor seals varied depending on the habitat: seals on sandbars were captured by deploying a seine net from a fast-moving boat; those on beaches were approached using four-wheel-drive vehicles and captured using a net; seals in the water were entangled in 25 cm mesh stretch gill nets. Harbor seals that were sampled from the Atlantic coast of USA ($n = 8$) were animals that stranded alive between 1987–93 and were blood sampled on admission to a rehabilitation facility (Table 1). In each case, seals were caught and restrained and blood was collected from the vertebral extradural vein into untreated glass tubes and allowed to clot for several hours (Geraci and Lounsbury, 1993). The serum was separated by centrifugation and stored at −20 C or lower. The ages of the harbor seals was determined by counting growth layer groups in thin longitudinal sections of canine teeth (Mansfield and Fisher, 1960). Females < 3-yr-old and males < 6-yr-old were considered juveniles (Boulva and

McLaren, 1979). Animals for which teeth were not available or were live-captured were aged on the basis of standard length. Females ≥130 cm and males ≥150 cm were considered adults (MacLaren, 1993).

Gray seals (*Halichoerus grypus*) (n = 255) on the Atlantic coast of North America were sampled for blood from December through February from 1991 to 1997. Samples were obtained from animals in breeding colonies on Amet Island and Sable Island (Nova Scotia, Canada), on the Northumberland Strait pack ice in the southern Gulf of St. Lawrence, and in the St. Lawrence Estuary (Table 1). Whelping areas on the ice were located by helicopter and seals were approached on foot, captured in nets, and manually restrained for sampling as described for harbor seals. Gray seals on land were approached on foot or by all-terrain vehicles and captured in nets prior to sampling. Hunter-killed animals were sampled by cardiac puncture after death. Ages were determined by counting growth layer groups in thin longitudinal sections of canine tooth dental annuli (Mansfield, 1991) or incisor teeth (Bernt et al., 1996). Gray seals are sexually mature at six years for males and four years for females (Hammill and Gosselin, 1995). Live-captured animals or animals for which no teeth were available were aged on the basis of standard length (adult males at ≥190 cm and adult females at ≥170 cm (MacLaren, 1993)).

Free-ranging harp seals (*Phoca groenlandica*) (n = 453) were sampled between 1988–1997 while hauled out on pack ice in the St. Lawrence Estuary, Gulf of St. Lawrence, and off the southeast coast of Newfoundland (Table 1). After breeding areas on the ice were located by helicopter, seals were approached on foot, captured in nets, and manually restrained. Blood was collected from either the tarsal venous plexus or the vertebral extradural vein and processed the same way as described for the harbor and gray seal samples. Some samples were also taken from hunter killed animals. Ages were estimated by counting growth layer groups in transverse sections of canine teeth (Bowen et al., 1993). Animals three years of age and less are sexually immature and were classified as juveniles (Sergeant, 1991; Sjare et al., 1996). Animals for which teeth were not available were aged on the basis of standard length (animals at ≥160 cm were considered adults) (MacLaren, 1993).

Capture and sampling of free-ranging hooded seals (*Cystophora cristata*) (n = 204) from the Gulf of St. Lawrence, the St. Lawrence Estuary, and Sable Island between 1988–97 was carried out in the same way as for the harp seals (Table 1). The five samples from Newfoundland were from hunter killed animals. One animal from the St. Lawrence Estuary, and two from the Gulf of St. Lawrence were found dead. Age was estimated from body length measurements (McLaren, 1993) or by counting transverse sections of canine teeth (Øritsland, 1975). Females < 3-yr-old and males < 4-yr-old were classified as juveniles (Øritsland, 1975).

Cetaceans

Beluga (*Delphinapterus leucas*) (n = 463), narwhal (*Monodon monocerus*) (n = 77) and bowhead whale (*Balaena mysticetus*) (n = 3) were sampled by Inuit or Department of Fisheries and Oceans personnel from 18 locations in Arctic Canada between 1984–97. Beluga ages were determined by counting growth layer groups in the dentine of longitudinally sectioned mandibular teeth (Brodie, 1969). Females less than five and males less than seven years old were classified as juveniles (Brodie, 1971). No valid method exists for determining the age of narwhal.

Blood samples were obtained from 19 live-stranded pilot whales (*Globicephala* sp.) between 1986 and 1994 from the northeastern coast of the USA. Twenty-five beach cast beluga, one minke whale (*Balaenoptera acutorostrata*), two northern bottlenose whales (*Hyperodoon ampullatus*), two harbor porpoises (*Phocoena phocoena*) and two Atlantic white-sided dolphins (*Lagenorhynchus acutus*) from the St. Lawrence Estuary were sampled between 1991–96 (Table 2). In addition, two beach cast Atlantic white-sided dolphins and one harbor porpoise were blood sampled from the Gulf of St. Lawrence in 1993 and 1994 (Table 2). All of the beached cetaceans were found dead.

Antibody assays

Antibodies to *Brucella abortus* antigens were tested using two competitive enzyme-linked immunosorbent assays (C-ELISA's) (Nielsen et al., 1992, 1995). These tests have been used previously to demonstrate the presence of *Brucella* antibodies in ringed seal and walrus blood samples (Nielsen et al., 1996). They can distinguish between *Brucella*-specific antibodies and antibodies elicited by other related gram-negative bacteria, even when using whole blood samples from a variety of animal species (Nielsen et al., 1992).

The O-chain tests were done as follows. Purified *B. abortus* O-polysaccharide, conjugated with poly-L-lysine was passively attached to the wells in a polystyrene 96 well plate and diluted test serum added (1:50). Attachment of antibody to the immobilized antigen was detected

TABLE 2. Scrum antibody prevalences to *Brucella* spp. in eight species of cetaceans from North America.

Location	Coordinates	Year	Species	Number	Positive[a]
St. Lawrence Estuary	47°00′N, 71°00′W to 49°30′N, 67°20′W	1994–95	Harbor porpoise[b]	2	0
		1996	Minke whale[b]	1	0
		1994	Northern bottlenose whale[b]	2	0
		1991, 94	Atlantic white-sided dolphin[b]	2	0
		1993–96	Beluga[b]	25	4 (16.0)[a]
Gulf of St. Lawrence	46°00′N, 59°40′W to 49°30′N, 67°20′W	1994	Harbor porpoise[b]	1	0
		1993–94	Atlantic white-sided dolphin[b]	2	0
US Atlantic Coast	45°00′N, 67°00′W to 40°30′N, 74°00′W 41°51′N, 70°00′W to 41°39′N, 70°42′W 40°59′N, 66°24′W	1986, 90, 94	Pilot whales[b]	19	0
Nunavik	56°32′N, 76°33′W to 58°41′N, 65°57′W to 63°20′N, 77°55′W	1994–95	Beluga	14	5 (35.7)
Hudson Bay					
Arviat	61°07′N, 94°04′W	1984, 86, 87	Beluga	69	5 (7.2)
Coral harbor	64°08′N, 83°10′W	1993	Beluga	11	0
Repulse Bay	66°32′N, 86°15′W	1996	Bowhead whale	1	0
		1993	Narwhal	6	0
Sanikiluaq	56°32′N, 79°14′W	1993–94	Beluga	30	0
Baffin Island					
Arctic Bay	73°02′N, 85°10′W	1986–87	Narwhal	3	0
Cape Dorset	64°14′N, 76°32′W	1990	Beluga	4	0
Lake harbor	65°51′N, 69°53′W	1987–94	Beluga	66	2 (3.0)
Iqaluit	63°45′N, 68°31′W	1986–94	Beluga	50	1 (2.0)
		1993–94	Narwhal	25	4 (16)
Pangnirtung	66°09′N, 65°43′W	1986–94	Beluga	83	5 (6.0)
Pond Inlet	72°42′N, 77°59′W	1992, 94	Narwhal	25	1 (4.0)
High Arctic					
Grise Fjord	76°25′N, 82°54′W	1984, 87	Beluga	27	4 (14.8)

TABLE 2. Continued.

Location	Coordinates	Year	Species	Number	Positive[a]
Foxe Basin					
Hall Beach	68°46′N, 81°13′W	1994	Narwhal	18	0
Igloolik	69°23′N, 81°48′W	1994	Bowhead whale	1	0
Mackenzie Delta					
East Whitefish	69°25′N, 133°36′W	1993–94	Beluga	24	1 (4.2)
Hendrickson Island	69°30′N, 133°36′W	1993–95	Beluga	53	1 (1.9)
Husky Lakes	69°15′N, 132°30′W	1989	Beluga	27	0
Shingle Point	60°00′N, 137°25′W	1993	Beluga	5	0
		1996	Bowhead whale	1	0
Grand total				597	33 (5.5)

[a] Number positive (percent positive).
[b] Stranded animals.

by addition of a mouse monoclonal antibody specific for the O-polysaccharide conjugated with horseradish peroxidase to the wells containing the O-chain antigen at the same time as the test serum.

In the second assay, purified smooth lipopolysaccharide (s-LPS), also isolated from *B. abortus*, was also allowed to passively attach to the wells of a 96 well plate. The test serum (1:10) and a monoclonal antibody to *B. abortus* O-polysaccharide (M84) were added to the wells. Monoclonal antibody binding was detected using goat anti-mouse IgG conjugated to horseradish peroxidase. For both tests, enzyme substrate and chromogen were added and the colored end product was measured photometrically. Test serum containing *Brucella*-specific antibodies would compete with the monoclonal antibody for antigenic sites thereby inhibiting the binding of those antibodies and resulting in diminished color development. For the O-chain assay a threshold of 20% or greater inhibition of competing antibody was adopted as a positive result but a threshold of 30% or greater inhibition was adopted for the M84 assay. Both thresholds were selected on the basis of results obtained with bovine sera (Nielsen et al., 1995). Bovine control sera (strong positive, weak positive, and a negative) were included in each plate. Marine mammal test sera, that met or exceeded this threshold were considered to have been previously exposed to *Brucella* spp. and were considered positive.

All hunter-killed animals were butchered and examined for abnormalities or signs of disease by hunters or Department of Fisheries and Oceans personnel prior to consumption.

Frequencies of seropositive animals were compared by the Chi-square goodness of fit test where possible. Statistical analyses were carried out with one or two degrees of freedom, depending on the number of parameters being compared (Snedecor and Cochran, 1967).

RESULTS

Overall, 3.8% (94 of 2,470) of the marine mammals sampled were positive in either one or both of the C-ELISA's (Table 3). Positive animals were identified in all five species of phocids tested including samples from the Atlantic, Pacific, and Arctic oceans. The overall prevalence of pinnipeds with antibodies to *Brucella* spp. was 3.3% (61 of 1873) (Table 3). No significant differences in antibody prevalence were found with respect to sex ($\chi^2 = 0.01$,

TABLE 3. Prevalence of *Brucella* spp. binding antibodies in fourteen species of North American marine mammals.

Species	Number tested	O-chain	M84	Number positive
Halichoerus grypus	255	1 (0.3)[a]	10 (3.9)	10 (3.9)
Phoca vitulina	163	9 (5.5)	21 (12.9)	21 (12.9)
Cystophora cristata	204	5 (2.4)	9 (4.4)	10 (4.9)
Phoca groenlandica	453	6 (1.3)	7 (1.5)	8 (1.8)
Phoca hispida	628	1 (0.2)	7 (1.1)	7 (1.1)
Odobenus rosmarus	170	5 (2.9)	3 (1.8)	5 (2.9)
Delphinapterus leucas	488	25 (5.1)	26 (5.3)	28 (5.7)
Monodon monoceros	77	5 (6.5)	5 (6.5)	5 (6.5)
Balaena mysticetus	3	0	0	0
Phocoena phocoena	3	0	0	0
Globicephala melas	19	0	0	0
Balaenoptera acutorostrata	1	0	0	0
Lagenorhynchus acutus	4	0	0	0
Hyperdoon ampullatus	2	0	0	0
Total	2,470	57 (2.3)	88 (3.6)	94 (3.8)

[a] Number positive (percent positive).

$P > 0.05$) or age class ($\chi^2 = 0.01$, $P > 0.05$) among all pinnipeds (Table 4 and 5).

Five of 170 (2.9%) walrus had antibodies to *Brucella* spp. (Table 3). Positive walrus were identified from Hall Beach (3/67) and Igloolik (2/90) (Table 1). Sample numbers were relatively low from the other sample sites of Nunavik ($n = 4$), Grise Fjord ($n = 5$) and Resolute Bay ($n = 4$) (Table 1), and no seropositive walrus were identified from these locations.

Among seal species, harbor seals had the highest prevalence of antibody positive animals (21/163 or 12.9%) (Table 3). Positive harbor seals were identified from all sampling sites in the Atlantic and Pacific Oceans (Table 1). There were no significant associations between prevalence and sex ($\chi^2 = 0.41$, $P > 0.05$) (Table 4) or age ($\chi^2 = 0.02$, $P > 0.05$) (Table 3) or location (Atlantic versus Pacific) ($\chi^2 = 2.23$, $P > 0.05$) (Table 2).

Of 255 gray seals sampled between 1991 and 1997 from four sites in the western Atlantic, positive animals were identified from Amet Island (2/67 or 3.0%), Sable Island (4/36 or 11.1%) and the Gulf of St. Lawrence (4/129 or 3.1%) (Table 1). The overall prevalence of seropositive gray seals was 10/255 or 3.9% (Table 3).

Among 204 hooded seals sampled in 1989–97, seropositive animals were present from all locations (Table 1). Seven of 194 (3.6%) animals were positive from the Gulf of St. Lawrence. Positive animals were also recovered from Sable Island, Nova Scotia, Newfoundland, and the St. Lawrence Estuary though only 10 animals were tested from these sites (Table 1). The overall prevalence of seropositive hooded seals was 10/204 or 4.9% (Table 3).

None of the 89 harp seals from the St. Lawrence Estuary had antibodies to *Brucella* spp.; however, 5/269 or 1.8% of the animals from the Gulf of St. Lawrence and 3/95 or 3.1% animals from Newfoundland were positive (Table 1). The overall prevalence of seropositive harp seals was 8/453 or 1.8%.

Six hundred twenty-eight ringed seals were sampled from six locations in Arctic Canada and Newfoundland. Positive samples were identified from Pangnirtung (6/208 or 2.9%) and Newfoundland (1/18 or 1.0%) (Table 1), making the overall prevalence (7/628 or 1.1%) (Table 3). No seropositive animals were identified from 276 seals from Arviat.

Among cetacean samples, only narwhal from (Iqaluit (4/25) and Pond Inlet (1/

TABLE 4. Prevalence of *Brucella* spp. binding antibodies in fourteen species of North American marine mammals.

Species	Female	Positive	Male	Positive	Unknown	Positive	Total	Total positive
Halichoerus grypus	150	4	98	6	7	0	255	10
Phoca vitulina	77	11	84	9	2	1	163	21
Cystophora cristata	145	9	59	1	0	0	204	10
Phoca groenlandica	306	6	147	2	0	0	453	8
Phoca hispida	155	2	210	5	263	0	628	7
Odobenus rosmarus	40	1	72	3	58	1	170	5
Delphinapterus leucas	190	15	261	12	37	1	488	28
Monodon monoceros	24	2	32	3	21	0	77	5
Balaena mysticetus	1	0	2	0	0	0	3	0
Phocoena phocoena	1	0	2	0	0	0	3	0
Globicephala melas	14	0	4	0	1	0	19	0
Balaenoptera acutorostrata	1	0	0	0	0	0	1	0
Lagenorhynchus acutus	1	0	2	0	1	0	4	0
Hyperdoon ampullatus	1	0	1	0	0	0	2	0
Total	1,106	50	974	41	390	3	2,470	94

25)), free-ranging Arctic beluga and beach cast beluga from the St. Lawrence Estuary were identified as *Brucella* spp. reactors (Table 2). Prevalence for beluga and narwhal was similar, 5.7% for beluga (28 of 488) and 6.5% for narwhal (5 of 77) (Table 3). No significant differences in antibody prevalence were detected among beluga ($\chi^2 = 2.00$, $P > 0.05$) and narwhal ($\chi^2 = 0.02$, $P > 0.05$) (Table 4) with respect to sex but there was a significant difference among beluga with respect to antibody prevalence and age. Juvenile belugas were more likely to have antibodies to *Brucella* spp. than adults (Table 5) ($\chi^2 = 5.46$, $P < 0.05$).

Differences in response between seals and cetaceans were found when the results of the M84 and O-Chain assays were compared. When all pinniped species were compared with respect to the assay that gave a positive response, the M84 assay was more than twice as likely to identify a seropositive animal than the O-Chain assay ($\chi^2 = 10.71$, $P < 0.05$) (Table 3). The exception to that rule is the walrus where the O-Chain assay identified more seropositive animals than the M84 assay (Table 3). For cetaceans (narwhal and beluga), no significant difference exists between assays with respect to ability to identify seropositive animals ($\chi^2 = 0.02$, $P > 0.50$) (Table 3).

In no cases where samples were submitted and the animals subsequently identified as having antibodies to *Brucella* spp. were gross pathologies found that were consistent with brucellosis in terrestrial or marine mammals. Personnel qualified to make such diagnoses did not carry out examination of carcasses.

DISCUSSION

A preliminary serological survey of Atlantic walrus and ringed seals in the Canadian Arctic found evidence of exposure to a *Brucella*-like organism (Nielsen et al., 1996). The study reported here extends that survey to include a greater number of species and a geographical range from New England (USA) to British Columbia

TABLE 5. Prevalence of *Brucella* spp. binding antibodies in eight species of North American marine mammals.

Species	Juvenile	Positive	Adult	Positive	Age unknown	Positive	Total
Halichoerus grypus	76	1	170	9	9	1	255
Phoca vitulina	96	11	65	9	2	1	163
Cystophora cristata	36	1	168	9	0	0	204
Phoca groenlandica	145	2	308	6	0	0	453
Phoca hispida	104	2	247	4	277	1	628
Odobenus rosmarus	12	1	52	3	106	1	170
Delphinapterus leucas	126	13	306	13	55	2	488
Monodon monoceros	0	0	0	0	77	5	77
Total	595	31	1,316	53	526	11	2,438

(Canada) and including most of Arctic Canada. Though it is possible that positive serologic tests may be due to cross reactivity with other gram-negative species of bacteria. The tests are quite specific for *Brucella* in terrestrial animals (Nielsen et al., 1992), and therefore this explanation seems unlikely. More likely, there is a low level of infection in some species of marine mammals throughout this range. Of the six pinniped and two cetacean species for which ≥77 samples were tested, all had seropositive individuals. Furthermore, infection by a *Brucella*-like pathogen is not a recent phenomenon in that the earliest positive samples were collected in 1984 and these were among the earliest samples available for testing. This is similar to the situation in the eastern Atlantic where a dolphin serum sample collected in 1990, the oldest sample in the panel, was positive (Jepson et al., 1997).

The ELISA's used in this and the earlier survey are consistent and specific for *Brucella* spp. antigens (Nielsen et al., 1995, 1996). Although the antigenic specificity of antibodies measured by both assays differs, there was excellent concordance between the tests when used on cetacean sera. In contrast, assay concordance was significantly less for pinniped sera, suggesting that a different species or biovar of *Brucella* infects them. We found no seropositive ringed seals at Arviat (0/276) although five of 69 beluga from the same location were positive (Table 1 and 2). Indeed, phenotypic comparison of *Brucella* spp. isolates from a range of marine mammal species have found differences in carbon dioxide requirements and in surface antigens between isolates from cetaceans and those from seals (Ewalt et al., 1994; Foster et al., 1996; Clavareau et al., 1998). Testing the hypothesis that two or more species or biovars of *Brucella* spp. infects pinnipeds and cetaceans in North American waters awaits the isolation and characterization of organisms from beached or hunter-killed animals.

Given the temporal and spatial overlap in sampling of walrus and ringed seals between this survey and the previous report (Nielsen et al., 1996), it seems logical to combine the data sets to determine a more accurate estimate of antibody prevalence. Walrus in the Canadian Arctic are thought to be distributed in four discrete stocks (Born et al., 1995). The samples examined were obtained from three of those stocks: Foxe Basin (n = 226); Northern Hudson Bay-Hudson Strait-Southeastern Baffin Island-Northern Labrador (n = 4); and North Water (Baffin Bay-Eastern Canadian Arctic, n = 9). Seropositive walrus were present only in the Foxe Basin stock (12/226, 5.3%). However, it is likely that the small number of animals sampled from the two remaining stocks is not representative.

The ringed seal is the most wide spread and prevalent marine mammal in the Arctic (Stirling et al., 1981) and, including the previous survey (Nielsen et al., 1996), 857

samples were examined from the species range in Canada. The overall prevalence for all sites is low (1.9%) however, apart from one seropositive animal from Eureka and two from Holman (Nielsen et al., 1996) the remaining seropositive ringed seals all came from the eastern Baffin Island-northern Labrador region where the prevalence was 4.7%. The reason for this skewed prevalence distribution is presumably because ringed seals, although dispersed throughout the Arctic, tend to be focally concentrated in areas of preferred habitat, are highly territorial, and undertake only limited migrations (Smith, 1987; Smith and Hammill, 1981). Thus, a *Brucella*-like pathogen may be circulating among the ringed seals of eastern Canada but may only sporadically infect animals in other areas.

Among the remaining phocids, there was a considerable variation in antibody prevalence ranging from approximately 2% in harp seals to 21% in harbor seals from Vancouver Island. The differences between species may reflect a species difference in susceptibility to infection or in exposure to infected animals. Social behavior, haul-out patterns, or contact with other host species may influence the latter. In Britain, where surveys of harbor and gray seals were conducted independently both in Scotland and in England-Wales, there were marked differences in antibody prevalence between species and locations (Ross et al., 1994; Jepson et al., 1997). However, the general range of antibody prevalences was similar to those reported here for North American phocids.

The data set for cetaceans was more variable and included large numbers of samples from hunter-killed animals and more limited samples from stranded or beached animals. The only species from which antibody prevalence can be discussed with reasonable confidence are belugas and narwhal. For both, the seroprevalence was approximately 6% and equivalent to that reported for the pinniped species. By comparison, approximately 28% of stranded harbor porpoises were seropositive in British waters (Ross et al., 1996; Jepson et al., 1997). However, as with ringed seals, if belugas stocks are considered individually it is apparent that much higher seroprevalence occurs in the belugas from Nunavik and St. Lawrence than in those from other regions.

We cannot yet say what significance brucellosis has in terms of clinical disease or impaired reproductive capacity for marine mammals in the Canadian Arctic. Brucellosis in terrestrial mammals is known to cause reproductive failure either as a result of placentitis, mastitis or orchitis leading either to abortion, perinatal death, or infertility, respectively (Kennedy and Miller, 1993). An unclassified *Brucella* spp. was isolated from an aborted dolphin fetus in the USA (Ewalt et al., 1994) and several isolates have been recovered from marine mammal reproductive tissues in Scotland (Foster et al., 1996). Impaired reproductive capacity may be detrimental for long-lived species with already low reproductive rates such as walrus, narwhal and belugas. Perhaps of greatest concern is the finding of 16% seroprevalence among the small St. Lawrence beluga stock that is already threatened by poor reproductive success (Sergeant, 1986). More extensive pathological examination of stranded or hunter-killed animals would be required to determine whether or not brucellosis is causing clinical disease that could be impairing reproductive success in these species.

Based on a survey of stranded animals in British waters, no significant difference in antibody prevalence was found with respect to age or sex (Jepson et al., 1997). Our study generally concurs with that finding except in the case of beluga whales in which juveniles were more likely than adults to have *Brucella* spp. antibodies. This is contrary to the situation in cattle and in caribou where animals up to the age of puberty are relatively refractory to infection (Kennedy and Miller, 1993; Ferguson, 1997). Too little is known of the pathogenesis, routes of infection, inherent

resistance, and virulence of the organism in belugas to speculate on why this age difference in seroprevalence occurs. However, one possible explanation is that chronic mastitis results in persistent shedding of bacteria into milk so that successive calves from infected females are exposed and seroconvert. However, persistent infection may not develop in all exposed calves and those that recover may lose their antibody titer with time.

Brucellosis in caribou, caused by *Brucella suis* biovar 4, has long been recognized as a threat both to Arctic wildlife and to aboriginal people exposed to infected meat (Huntley et al., 1963; Tessaro and Forbes, 1986; Forbes, 1991). Indeed, the cases of human brucellosis among Inuit on Baffin Island have been increasing in recent years (Ferguson, 1997). The zoonotic potential of the newly discovered marine mammal *Brucella* spp. strains is unknown but given that most of the known strains are human pathogens, it would be prudent to regard the marine mammal strains as such until proven otherwise (Davis, 1990; Carter et al., 1997). An isolate from a marine mammal has caused brucellosis in a British researcher (Brew et al., 1999). Ringed seals, walrus, beluga and narwhal are an important constituent of the Inuit diet and exposure to *Brucella* organisms could occur through dressing carcasses or by consuming raw meat.

In summary, the present study indicates that uncharacterized *Brucella* spp. are associated with at least some of the marine mammal species in the coastal zones of North America. The significance of this infection in terms of reproductive impairment or potential for zoonotic disease is unknown. However, it is likely that that there is risk to people who come in contact with marine mammals that have stranded and are likely to be ill, or those who consume hunted animals. Caution and good hygienic practices are advised in either situation. Furthermore, centers involved in marine mammal rehabilitation ought to routinely screen for *Brucella* spp. as a standard operating procedure.

Our data suggest that marine mammals inhabiting the coastal waters of Atlantic, Pacific and Arctic Canada as well as the United States have been infected with *Brucella* spp. or a bacteria that cross reacts with *Brucella* spp. antigens in the two C-ELISA's that were used. Since the antigens used in the preparation of the reagents for the tests are unique to the genus *Brucella* (Nielsen et al., 1995) it is more likely that the positive animals identified were indeed infected with species of *Brucella.*

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