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Serologic Evidence of *Brucella* spp. Exposure in Atlantic Walruses (*Odobenus rosmarus rosmarus*) and Ringed Seals (*Phoca hispida*) of Arctic Canada

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ABSTRACT. The first presumptive evidence of *Brucella* infection in marine mammals of Arctic Canada is reported. Blood samples were collected from 248 ringed seals (*Phoca hispida*) and 59 Atlantic walrus (*Odobenus rosmarus rosmarus*) from eight locations in the Canadian Arctic between 1987 and 1994. A competitive enzyme-linked immunosorbent assay (C-ELISA), using a specific monoclonal antibody to *Brucella* spp. cell wall components, was used to detect anti-*Brucella* spp. antibodies in the samples. Sera from ten seals and seven walruses exceeded the C-ELISA threshold that indicates that cattle have been exposed to *Brucella* spp. Five of the positive walrus sera were suitable for the tube agglutination test. All five were confirmed positive using this test. Although the bacterium has not yet been identified, it appears that a *Brucella* sp. or a *Brucella*-like bacterium may be enzootic in these species in the Canadian Arctic. It is also possible that the very low prevalence of antibodies in ringed seals and the seemingly random distribution of seropositive animals may indicate a sporadic infection from another enzootically infected phocid or predator (e.g., Arctic fox *Alopex lagopus*). Or perhaps, limited epizootics may have occurred in the areas where seropositive seals were found. A similar situation could also exist in the walrus of Foxe Basin.

Key words: walrus, *Odobenus rosmarus rosmarus*, ringed seal, *Phoca hispida*, *Brucella*, brucellosis, competitive ELISA, tube agglutination test.

RÉSUMÉ. On rapporte la première preuve par inférence d'une infection des mammifères marins de l'Arctique canadien par le bacille *Brucella*. Entre 1987 et 1994, on a effectué des prélèvements sanguins sur 248 phoques annelés (*Phoca hispida*) et 59 morses de l'Atlantique (*Odobenus rosmarus rosmarus*) à huit endroits dans l'Arctique canadien. On a employé la technique immuno-enzymatique par compétition ÉLISA, en se servant d'un anticorps monoclonal spécifique aux composants de la paroi cellulaire de *Brucella* spp, afin de détecter les anticorps anti-*Brucella* sp dans les prélèvements. Les sérums de dix phoques et de sept morses dépassaient le seuil d'ÉLISA qui indique que la population animale a été exposée à *Brucella* spp. Cinq des sérums positifs de morses se prêtaient à l'épreuve d'agglutination en tube, et tous se sont révélés positifs. Bien qu'on n'ait pas encore identifié la bactérie, il semble que *Brucella* sp ou une bactérie semblable à *Brucella* puisse être enzootique à ces espèces dans l'Arctique canadien. Il est également possible que la faible fréquence globale d'anticorps chez le phoque annelé et la distribution apparemment erratique des animaux séropositifs soient l'indice d'une infection sporadique communiquée par un autre phocidé infecté par proximité dans le milieu ou encore par un prédateur (p. ex., le renard arctique *Alopex lagopus*). Ou bien des épizooties se seraient produites dans les régions où l'on a trouvé des phoques séropositifs. Une situation semblable pourrait aussi se retrouver chez le morse du bassin de Foxe.

Mots clés: morse, *Odobenus rosmarus rosmarus*, phoque annelé, *Phoca hispida, Brucella*, brucellose, ÉLISA par compétition, épreuve d'agglutination en tube

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INTRODUCTION

Brucella is a genus of gram-negative bacteria whose members can infect both domestic and wild mammals. The resulting disease is known as brucellosis. There are six recognized species of *Brucella*. Three species can occur as one of several biovars or strains. All but two species are pathogenic in humans (Mayfield et al., 1990). In domestic mammals, brucellosis causes abortions and other reproductive disorders. In humans, these pathogens are often associated with influenza-like symptoms, although other complications may occur.

Brucella spp. also occur in a number of wildlife species in Arctic Canada. Rangiferine brucellosis, the most common form of brucellosis in the Arctic, is caused by *Brucella suis* biovar 4. The disease primarily affects reindeer and caribou (*Rangifer tarandus*) (Neiland et al., 1968), moose (*Alces alces*) (Dietrich et al., 1991; Honour and Hickling,

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1993), and muskox (*Ovibos moschatus moschatus*) (Gates et al., 1984). *Brucella suis* biovar 4 has also been isolated from animals that consume infected ungulate carcasses, such as wolves (*Canis lupus*), dogs (*C. familiaris*), red foxes (*Vulpes vulpes*), and grizzly bears (*Ursus arctos*) (Neiland, 1970, 1975). It has been isolated in Siberia and Alaska as well as in Canada (Neiland et al., 1968). Human brucellosis has been reported from a number of locations in Arctic Canada (Tessaro and Forbes, 1986).

There have been few studies of *Brucella* spp. in marine mammals. There is preliminary evidence of *Brucella* spp. in a few stranded harbour seals (*Phoca vitulina*), harbour porpoises (*Phocoena phocoena*), and a common dolphin (*Delphinus delphis*) from the Scottish coast, although the bacterium was not identified (Ross et al., 1994). A culture of *Brucella* sp. isolated from an aborted bottlenose dolphin (*Tursiops truncatus*) fetus from California did not belong to any known species or biovars (Ewalt et al., 1994). An initial survey in Alaska found no evidence of *Brucella* spp. in 12 Pacific walrus (*Odobenus rosmarus divergens*) (Seagers et al., 1995).

There have been no reported surveys for *Brucella* spp. in marine mammals in the Canadian Arctic. However, these mammals are an important source of food in most communities. Their health and the impact of consuming arctic marine mammals on human health are, therefore, of utmost importance to Northerners. Here we report the first indirect evidence of *Brucella* spp. in Atlantic walrus (*Odobenus rosmarus rosmarus*) and ringed seals (*Phoca hispida*) from the Canadian Arctic.

METHODS

As part of ongoing marine mammal sampling programs (e.g., Garlich-Miller, 1994), blood, reproductive tracts and other tissues were collected and held frozen during Inuit subsistence hunts throughout the Canadian Arctic. Ringed seals were sampled between 1992 and 1994 at seven locations (Fig. 1). Walrus were sampled between 1987 and 1993 at two locations in Foxe Basin (Fig. 1). Usually, blood was collected within hours of death, frozen whole, and maintained at -20˚C before testing. For some samples, the serum was separated before initial freezing. Prior to testing, both blood and sera were centrifuged at 1000 × g for ten minutes.

The sera were tested for anti-*Brucella* spp. antibodies by two competitive enzyme-linked immunosorbent assays (C-ELISA) (Nielsen et al., 1992, 1995). The C-ELISAs are robust in this application. The tests allow the detection of *Brucella*-specific antibodies in a number of animal species and also distinguish between *Brucella* spp. and other related gram-negative bacteria, even when whole blood samples are used (Nielsen et al., 1992). Briefly, the C-ELISA assays involve the adsorption of *Brucella* spp. smooth lipopolysaccharide (s-LPS) antigen or polysaccharide antigen conjugated with poly-L-lysine to a solid plastic matrix. Diluted (1:50) test serum or blood and a mouse monoclonal antibody (MAB) specific for antigenic determinants of the polysaccharide are then added. The serum dilutions and antibody solutions were mixed simultaneously in a microtiter plate containing the bound antigen. The mixtures were incubated for one to two hours. In the first assay, the MAB was labelled with horseradish peroxidase. The subsequent chromagen mixture was added after the incubation step. In the second assay, goat anti-mouse IgG antibody conjugated to horseradish peroxidase was added. After incubation, enzyme substrate and a chromagen were added, and the subsequent coloured product was measured photometrically. If the test serum contained *Brucella*-specific antibodies, they competed with the MAB for antigenic sites, thereby inhibiting the binding of the MAB and the subsequent colour development. A threshold at 30% or greater inhibition of MAB binding was selected on the basis of results obtained with bovine sera (Nielsen et al., 1995). Sera which met or exceeded this threshold are believed to be indicative of previous exposure to *Brucella* spp. These sera will be referred to as positive. The C-ELISA positive sera were tested by the standard tube agglutination test (MacMillan, 1992). Only unhaemolyzed sera were suitable for this test, and a tube agglutination titer of 20 or greater was considered positive.

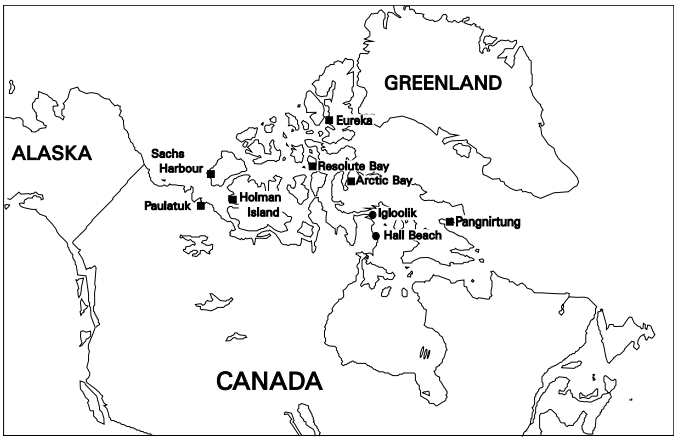

FIG. 1. Locations for collection of blood samples from ringed seals (*Phoca hispida*) (■) and walruses (*Odobenus rosmarus rosmarus*) (●) tested for evidence of exposure to *Brucella* spp.

Ages of the seals and walrus were estimated by counting dental annuli in the canine teeth (McLaren, 1958; Garlich-Miller et al., 1993). Reproductive tracts of adult females that had serologic evidence of exposure were thawed, dissected, and examined for gross evidence of reproductive failure or abnormalities.

RESULTS AND DISCUSSION

Approximately 4% (10 of 248) of the seals and 12% (7 of 59) of the walrus were positive in the C-ELISA tests (Table 1). Five of the seven positive walrus sera were suitable for confirmation using the tube agglutination test.

TABLE 1. Serum antibody prevalence of *Brucella* spp. in two species of marine mammals from Arctic Canada.

Species	Location		Dates	Number tested	Positive	
					No.	%
Ringed seal						
	Pangnirtung	65°22′N, 66°30′W	November '92 – February '93	75	7	9
	Arctic Bay	73°01′N, 85°07′W	April – June '93	38	0	0
	Resolute Bay	74°40′N, 95°00′W	June '93	8	0	0
	Eureka	80°00′N, 86°00′W	May – June '94	17	2	12
	Paulatuk	69°35′N, 123°40′W	August – September '93	9	0	0
			August – September '94	38	0	0
	Sachs Harbour	71°59′N, 125°15′W	July '93	3	0	0
	Holman Island	70°39′N, 101°31′W	May – July '93	31	0	0
			May – July '94	29	1	3
	All locations			**248**	**10**	**4**
Walrus						
	Igloolik	69°23′N, 81°40′W	July – August '87	16	1	6
			July – August '88	36	6	17
			July – August '93	3	0	0
	Hall Beach	68°47′N, 81°13′W	July – August '93	4	0	0
	All locations			**59**	**7**	**12**

All were positive at dilutions of 1:20, 1:40, or 1:80. None of the seal samples were suitable for tube agglutination analysis since they were badly haemolyzed. Broughton et al. (1970) found *Brucella* spp. agglutinating antibody titers of 1:25 or greater in 4.37% (14 of 320) of the caribou from Kaminuriak, Northwest Territories and in 8.74% (148 of 1692) of sera from reindeer in the Mackenzie Delta. These data seem consistent with the prevalences we report from seals and walrus.

The seropositive ringed seals came from three widely distant sites (Pangnirtung, Eureka, and Holman Island). These areas are separated by 1250 to 2100 km, and no seropositive seals have been detected at locations between them. A chi-square goodness-of-fit test was performed on ringed seal data for locations with more than 15 samples. Results indicated the observed frequency of positive seals was significantly different among sites ($p < 0.05$, 1 d.f.). This difference was apparently due to the lack of positive sera from Arctic Bay and Paulatuk. Among the sites with positive animals, there were no significant differences from the expected frequency ($p > 0.05$, 1 d.f.). However, as the expected prevalence values were less than five for all cells, these statistical results should be viewed with caution. All the walrus samples originated from one population in the Foxe Basin (Richard and Campbell, 1988).

Seropositive walrus ranged in age from 10 to 13 years and five of the seven were female, while seropositive ringed seals ranged from 0 to 18 years and seven of the ten were female. However, there were too few data to examine age or sex differences statistically. The highest frequency of positive reactions for walrus was in 1988. However, there were too few data to test for temporal trends.

Reproductive status was available for three seropositive female walrus and one of the positive female ringed seals. All appeared normal. Another seropositive adult ringed seal was nursing an apparently healthy pup when collected. One researcher ate uncooked parts of one of the seropositive seals from Eureka and tested negative for *Brucella* spp. exposure approximately one year later. Presumably the Inuit who subsist on seals and walrus are exposed to this bacterium too. However, there is no evidence suggesting that prevalence of brucellosis in humans who live where we found serologic evidence of *Brucella* spp. in seals and walruses is different from its prevalence in other areas in the Northwest Territories (A. Corriveau, Territorial Epidemiologist, Government of the Northwest Territories, pers. comm. 1996).

There are several possible explanations for the serologic test results obtained in this survey: (1) The agent which elicited the antibody response may be a known strain of *Brucella* that may or may not be pathogenic in marine mammals. (2) The agent may be a new strain of *Brucella*. (3) Positive serologic test results may be due to some other bacterium that mimics *Brucella* in the ELISA and agglutination tests. However, these tests are quite specific for *Brucella* in terrestrial mammals (Nielsen et al., 1992), and we think that this explanation is unlikely.

The very low prevalence of anti-*Brucella* spp. antibodies in ringed seals and the random distribution of seropositive animals may also indicate sporadic infection, perhaps from another enzootically infected phocid or from a predator such as the Arctic fox (*Alopex lagopus*). Alternatively, limited epizootics may have occurred in the areas where seropositive seals were found. Walruses could also have been infected in a similar manner. Research is presently underway to isolate the responsible bacterium. In addition, we hope to determine the number and geographic range of other marine mammal species that have been exposed.

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