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Brucellosis in Ringed Seals and Harp Seals from Canada

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ABSTRACT: A novel *Brucella* sp. was isolated from lymph nodes of four ringed seals (*Phoca hispida*) collected near Pangnirtung (Baffin Island, Canada) in January and February 1995 and in one harp seal (*Phoca groenlandica*) collected near the Magdalen Islands (Gulf of St. Lawrence, Canada) in March 1996. Bacteriological characteristics were the same for all five isolates. The colonies were typical of *Brucella* spp., but took 2 to 5 days longer than the traditional species to appear on primary isolation media. Biotyping results did not match any of the known biovars of *Brucella,* but were similar to isolates of the genus *Brucella* previously reported from marine mammals inhabiting other areas of the northern hemisphere. This is the first confirmed report of brucellosis in marine mammals from Canada, and the first report of this organism in ringed and harp seals.

Key words: *Brucella* sp., brucellosis, Canada, harp seal, marine mammals, *Phoca groenlandica, Phoca hispida,* ringed seal.

Brucellosis in marine mammals was first described in 1994 when a bacterial isolate from the aborted fetus of a bottlenose dolphin (*Tursiops truncatus*) was characterized as a nontypical *Brucella* sp. (Ewalt et al., 1994). Since that time, *Brucella*-like organisms have been found in other bottlenose dolphins (Miller et al., 1999), a common dolphin (*Delphinus delphis*) (Ross et al., 1994), harbour porpoises (*Phocoena phocoena*) (Ross et al., 1994; Foster et al., 1996), an Atlantic white-sided dolphin (*Lagenorhynchus acutus*) (Foster et al., 1996), striped dolphins (*Stenella coeuleoalba*) (Foster et al., 1996), a minke whale (*Balaenoptera acutorostrata*) (Clavareau et al., 1998), common (harbour) seals (*Phoca vitulina*) (Ross et al., 1994; Foster et al., 1996), a hooded seal (*Cystophora cristata*) (Foster et al., 1996), a grey seal (*Halichoerus grypus*) (Foster et al., 1996), a Pacific harbour seal (*Phoca vitulina richardsi*) (Garner et al., 1997), and a European otter (*Lutra lutra*) (Foster et al., 1996). These reports suggest that *Brucella* sp. infection is common in marine mammals, at least in the northern hemisphere. In 1996, a serological survey of marine mammals from the Canadian arctic detected anti-*Brucella* antibodies in Atlantic walruses (*Odobenus rosmarus rosmarus*) and ringed seals (*Phoca hispida*) (Nielsen et al., 1996). Confirmation of infection by bacterial isolation was not reported at that time. The purpose of this report is to document the recovery of a *Brucella* sp. from Canadian seals and to provide a brief description of the isolates.

Eight seals (six ringed seals, one harp seal (*Phoca groenlandica*), and one grey seal) were selected for culture based on the presence of anti-*Brucella* antibodies in their sera as determined using a competitive ELISA conducted as previously described (Nielsen et al., 1996). The threshold for detection was established using bovine sera and was set at ≥30% inhibition of monoclonal antibody binding (Nielsen et al., 1995). Sera from all seals met or exceeded that value. All seals appeared grossly normal at post mortem examination. Bacteriological culture and biotyping were done as previously described (Alton et al., 1988). Isolates were made from pooled lymph node samples from each of four ringed seals collected near Pangnirtung (Baffin Island, Canada; 66°09′N, 65°43′W) in January and February 1995, and from one harp seal collected near the Magdalen Islands (Gulf of St. Lawrence, Canada; 47°23′N, 61°52′W) in March 1996.

TABLE 1. Characteristics of isolates of *Brucella* sp. made from four ringed seals and one harp seal from Canada.

Characteristics	Result
General	
CO_2 requirement for primary culture	Yes
Good growth on media	5 to 7 days
H_2S produced	No
Catalase present	Yes
Urease present/reaction time	Yes/1.25 hr
Serum requirement for growth	No
Growth in media containing the following:	
Thionin 1:25,000	Yes
B. fuchsin 1:25,000	Yes
Thionin bue 1:500,000	Yes
Safranin O 1:5,000	Yes
Penicillin 5.0 μg/ml	Yes
Erythritol 1.0 μg/ml	Yes
Erythritol 2.0 μg/ml	Yes
Lysis by *Brucella* bacteriophages at routine test dilution.	
Tbilisi[a]	No
Firenze	No
S708	No
Weybridge	Inconclusive
Berkley2	Variable
Rough	No
Rough Ovis	Yes
Rough canis	No
Delta	No
Me75	No
Surface antigens	
A antigen	Yes
M antigen	No
R antigen	No

[a] Tbilisi phage did not cause lysis at routine test dilution or 10^4 routine test dilution.

The colonies of *Brucella* sp. from the seals were typical of *Brucella* spp., but took 7 to 10 days to appear on primary isolation media as opposed to the 3 to 5 days usually observed for *B. abortus*, *B. suis*, and *B. melitensis*. Biotyping results were the same for all five isolates and were typical for *Brucella* spp., but did not match any known biovar (Table 1).

The standard method used to identify the six presently accepted *Brucella* spp. and their biovars is based on CO_2 dependance, utilization of various substrates, susceptibility to dyes and antibiotics, phage lysis, and surface antigens (Alton et al., 1988). Data from previous studies indicated that the organism isolated from marine mammals is distinct from the known species of *Brucella* and may consist of two or more biovars (Ewalt et al., 1994; Foster et al., 1996; Garner et al., 1997; Clavareau et al., 1998; Miller et al., 1999). Differences in dominance of antigens between isolates from bottlenose dolphins and seals, and an isolate from a minke whale have been reported, as have differences in CO_2 dependance between seal and cetacean isolates (Ewalt et al., 1994; Foster et al., 1996; Garner et al., 1997; Clavareau et al., 1998). This suggests that some marine mammals may be the primary hosts for certain strains of marine

Brucella sp. and is consistent with host specificity observed among terrestrial biovars of *Brucella.* For instance, swine are the primary hosts for *B. suis* biovars 1 and 3, European hares (*Lepus capensis*) for *B. suis* biovar 2, reindeer (*Rangifer tarandus tarandus*) and caribou (*R. t. groenlandicus, R. t. pearyi*) for *B. suis* biovar 4, and rodents for *B. suis* biovar 5. Recent work with genomic fingerprinting suggested that dolphin isolates were genetically distinct from seal and porpoise isolates (Jensen et al., 1999). Molecular characterization of a *Brucella*-like bacterium recovered from a minke whale indicated that it was also a unique *Brucella* sp. and the results of traditional typing procedures indicated it was similar to isolates from porpoises (Clavareau et al., 1998). However, the whale isolate has not been compared with isolates from other marine mammals and may represent a unique group. The isolates reported here had characteristics consistent with the previously reported isolates from seals including CO_2 dependence and the presence of a dominant A antigen, although the genotype was not examined.

The effect of this organism on the health and reproductive capacity of seals and other marine mammals is unknown. Serological surveys indicate that healthy marine mammals have antibodies to *Brucella* spp. (Nielsen et al., 1996; Ross et al., 1996; Tryland et al., 1999), and the isolates from this study all came from grossly normal animals. These novel *Brucella* spp. have been recovered from moribund and dead marine mammals but are not usually associated with visible lesions (Foster et al., 1996). The gross lesions reported in association with the organism have been subcutaneous lesions in the skin of two dolphins and one porpoise (Foster et al., 1996), placentitis in two captive dolphins following abortion (Miller et al., 1999), and a mineralized lung granuloma in a captive dolphin (Miller et al., 1999). The significance of *Brucella* spp. in dead, dying or aborting marine mammals is speculative and its role in the pathogenesis of lesions is unknown. Lungworms (*Parafilaroides* sp.) containing intrauterine *Brucella*-like organisms have been described in an infected Pacific harbour seal and these nematode parasites may play a role in maintaining and transmitting brucellosis among marine mammals (Garner et al., 1997).

Butchering, consumption of raw meat, and post mortem examinations provide ample opportunity for human exposure to *Brucella* sp. from marine mammals. Despite this, there are no reported field cases of human infection with this organism. Bacterial recoveries from cases of human brucellosis in the arctic have all been *B. suis* biovar 4 with strong epidemiological links to infected reindeer or caribou (Dieterich, 1981; Forbes, 1991). There is one report of a laboratory acquired infection with marine mammal *Brucella* sp. in a human which caused transient symptoms with no relapses following treatment (Brew et al., 1999). Although available information suggests that *Brucella* sp. from marine mammals is not a significant pathogen for humans, appropriate precautions should be taken under conditions of potential exposure until more data are available.

In summary, a novel *Brucella* sp., similar to that described in marine mammals from Europe and the USA, is described for the first time from Canada and is the first report of brucellosis in ringed seals and a harp seal. A number of researchers have worked on the characterization and distribution of marine mammal isolates of *Brucella,* but the pathogenesis, epidemiology and significance of marine mammal brucellosis remains speculative. Knowledge of the mode of transmission and the pathogenesis, particularly as it relates to reproduction, is required to assess the effect of brucellosis on the population dynamics of marine mammals, and it is in these areas where future research should be focussed.

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