Brucella spp. in the western North Pacific and Antarctic cetaceans: a review

KAZUE OHISHI*, YOSHIHIRO FUJISE+ AND TADASHI MARUYAMA*

Contact e-mail: oishik@jamstec.go.jp

ABSTRACT

Brucella spp. has been reported in a variety of marine mammals worldwide. Serological and pathological studies were conducted on *Brucella* spp. in the western North Pacific using samples from three whale species collected during 2000 under the second phase of the Japanese Whale Research Program under Special Permit in the Western North Pacific (JARPN II). Serum samples from 40 common minke whales (*Balaenoptera acutorostrata*), 43 Bryde's whales (*B. edeni*) and 4 sperm whales (*Physeter macrocephalus*) were assessed with agglutination testing designed for *B. abortus* detection. *Brucella*-specific serum antibodies were detected in 38% of common minke whale samples. A lower prevalence (9%) of the antibody was observed for the Bryde's whale samples, whereas no specific antibody against *Brucella* was observed for the four sperm whales. Serum samples from 104 Antarctic minke whales (*B. bonaerensis*) collected under the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA) were analysed, and no *Brucella*-specific antibodies were detected. Granular lesions with caseation and mineralisation were found in 35% (13 males and one female) of 40 minke whale gonads. Similar lesions were also observed in the gonads of one male and one female Bryde's whale. These gonad lesions were not found in 440 Antarctic minke whales and five sperm whales, despite the thorough examination conducted for reproduction studies. Histopathological studies showed that the lesions consisted of epithelioid cells, multinucleated giant cells and had an infiltration of lymphocytes. DNA fragments were amplified by PCR using specific primers from ten of 22 abnormal testis tissues collected from common minke whales. The DNA sequences had IS*711* transposable elements downstream of *bp26*, characteristic of marine strains of *Brucella spp*. The gene structure of *omp2*, and specific PCR products for seal strains, showed similarity to Atlantic seal strains rather than Atlantic whale strains. This showed that classification based on marine mammal host species, *B. cetacea* and *B. pinippedia* is not appropriate. Considering the zoonotic nature of the genus *Brucella*, the crews and researchers who have had frequent contact with whales were serologically examined and were found to have no health issues associated with this agent. No *Brucella*-specific antibody was detected in the sera from 51 persons examined in 2001, nor from 103 examined issues in 2003.

KEYWORDS: DISEASES, *BRUCELLA;* COMMON MINKE WHALE; ANTARCTIC MINKE WHALE; BRYDE'S WHALE; SPERM WHALE; REPRODUCTION; GENETICS; IMMUNOLOGY; BIOMARKERS; JARPA; JARPNII; PACIFIC OCEAN; ANTARCTIC OCEAN; SCIENTIFIC PERMITS; SURVEY-VESSEL

INTRODUCTION

Brucella, a genus of gram-negative bacteria, is a causative agent of brucellosis, a worldwide zoonotic disease (Corbel and Banai, 2005). Brucellosis has been studied in domesticated mammals, such as cattle, sheep, and pigs, and is known to cause reproductive disorders such as abortions in infected animals. Six species of *Brucella* are recognised from terrestrial animals: *Brucella abortus*; *B. melitensis*; *B. suis*; *B. ovis*; *B. canis*; and *B. netomae*, principally associated with cattle, sheep, pigs, goat, dogs and desert rats, respectively (Corbel and Banai, 2005). *Brucella* species may cause abortion, orchitis, epididymitis, arthritis, and/or spondylitis in these domesticated animals, and result in economic losses. *Brucella* spp. has also been isolated from a variety of terrestrial wildlife mammal species such as bison, elk, buffalo, reindeer, caribou, moose, feral swine, wild boars, foxes and hares (Davis, 1990; Rhyan, 2000). The first reports of *Brucella* spp. infection of marine mammals appeared in 1994 after isolation of the bacterium from free-ranging species of seals and cetaceans (Ewalt *et al*., 1994; Ross *et al*., 1994). Phenotypic and bacteriological characteristics of the marine strains differed from those of the six terrestrial varieties (Clavareau *et al*., 1998; Jahans *et al*., 1997). Molecular biological analysis has further supported the hypothesis that marine strains are different from terrestrial ones (Clavareau *et al*., 1998; Cloeckaert *et al*., 2000; Cloeckaert *et al*., 2003; Cloeckaert *et al*., 2001). These studies have suggested that *Brucella* marine strains were not recently introduced from terrestrial animals and might have evolved with marine mammals (Foster *et al*., 2002; Foster *et al*., 2007).

Recent extensive studies have revealed that *Brucella* infection or seroconversion occurs in a variety of marine mammals. Seroepidemiological analysis is useful in investigating the geographic distribution and host range of *Brucella* in marine mammals. Much of the information currently known is from the Northern Hemisphere, especially from waters around Europe and North America. Data from the western Pacific and Southern Hemisphere are very limited.

In addition to these serologic investigations, molecular analysis can identify the strains and provide information on bacterial identification, transmission and evolution. Here recent studies of serology, pathology and molecular biology of *Brucella* spp. in baleen whales inhabiting the western North Pacific and Antarctic Oceans are reviewed.

Serologic survey in whales

A serological study on *Brucella* spp. in the western North Pacific was conducted using samples of serum from whales collected in 2000 in sub-areas 7 and 9 (35°N, 141-170°E) under the second phase of the Japanese Whale Research Program under Special Permit in the Western North Pacific (JARPN II). Serum samples from 40 common minke whales (*Balaenoptera acutorostrata*), 43 Bryde's whales (*B. edeni*) and 4 sperm whales (*Physeter macrocephalus*) were assessed with agglutination testing using *B. abortus*.

*Japan Agency for Marine-Earth Science and Ecology (JAMSTEC), Research Program for Marine Biology and Ecology, Natsushima 2-15, Yokosuka, 237-0061 Japan.

+The Institute of Cetacean Research, Toyomi-cho 4-5, Chuo-ku, 104-0055 Japan.

Brucella-specific antibodies were observed in 38% of common minke whale samples (Ohishi *et al*., 2003). The details of the serological data of the common minke whales are summarised in Table 1. No significant difference was observed in sex, sexual maturity and sampling area, using 95% confidence intervals (Hughes-Hanks *et al*., 2005). A lower prevalence (9%) of the antibody was observed in Bryde's whale samples and no *Brucella*-specific antibodies were detected in the examined four sperm whale samples. Serum samples from 104 Antarctic minke whales (*B. bonaerensis*) obtained in 2000/01 in Antarctic areas V and VI-West (60-78°S, 130°E-145°W) under the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA), were also examined. In contrast to common minke whales in the North Pacific, no antibody was detected in the sera (Ohishi *et al*., 2003). These serological results suggest: (1) *Brucella* exposure occurred in the examined whale species in the western North Pacific in the 2000 survey; (2) a relatively higher exposure rate was observed in the common minke whale; and (3) Antarctic minke whales were not exposed to *Brucella* spp.

Histopathological study of the abnormal gonads

Lesions were observed in the testes and uterine endometrium in 35% (13 males and one female) of common minke whales in the JARPN II 2000 survey. The lesions were characterised as granular lesions with caseation and mineralisation (Ohishi *et al*., 2003). Similar lesions were found in epididymis and ovary in two mature Bryde's whales. Similar gonad lesions were not observed in 440 examined Antarctic minke whales and five examined sperm whales in the North Pacific. Histopathological studies showed that the granular lesions were associated with a proliferation of epithelioid cells or giant cells and the infiltration of lymphocytes (Ohishi *et al*., 2003). The lesions were negative for Ziehl-Neelsen staining, suggesting no mycobacteria in these lesions, which are known to induce similar lesions in lung tissue. No positive reactions with anti-*B. abortus* antibodies were observed in these lesions by immunostaining. However, *Brucella* specific fragments of DNA were detected from the gonad lesions by Polymerase Chain Reactions (PCR) with specific primers as described below.

Molecular biology of the Pacific whale *Brucella*

Recent molecular studies have enabled the identification of *Brucella* strains. Several molecular markers have been used to distinguish marine strains from terrestrial strains. The presence of transposable element IS*711* between the *bp26* gene and the Bru-RS1 palindrome element is unique for currently known marine isolates (Cloeckaert *et al*., 2000). Based on infrequent restriction site-polymerase chain reaction (IR-PCR), four kinds of DNA fragments (Fragment-I, II, III, and IV) are specific for currently known marine isolates (Cloeckaert *et al*., 2003). Fragment I (F-I) is specific for *Brucella* spp. from seals inhabiting the Atlantic Ocean (Atlantic seal strains), whereas Fragments-II, III, and IV (F-II, III, and IV) are specific for *Brucella* spp. from cetaceans inhabiting the Atlantic Ocean (Atlantic cetacean strains). The *omp2* gene encoding the outer membrane protein has been shown to be useful for molecular classification due to an appropriate polymorphism (Ficht *et al*., 1996). Terrestrial isolates have two gene copies, *omp2a* and *omp2b* at this locus, which share approximately 85% DNA sequence identity, except for *B. ovis* that has actually two *omp2a* genes. In marine *Brucella* strains, Atlantic seal strains possess *omp2a* and *omp2b*, whereas Atlantic cetacean strains have two *omp2b* genes (Cloeckaert *et al*., 2001).

DNA samples were obtained from granular testes of 22 common minke whales collected in sub-areas 7, 8, and 9 under JARPN II 2000 and JARPN II 2001 (Ohishi *et al*., 2004b; Ohishi *et al*., 2003). *Brucella* specific DNA fragments were detected by PCR with specific primers in ten of the 22 samples (Ohishi *et al*., 2004b).

*Insertion of IS*711 *transposable element downstream of* bp26

All ten *Brucella*-positive DNA samples from the testes of the North Pacific common minke whales contained IS*711* element downstream of the *bp26* (Ohishi *et al*., 2004b). This result suggests that *Brucella* spp. from North Pacific common minke whales have a close relationship to *Brucella* spp. from marine mammals in the North Atlantic.

Detection of marine-specific DNA fragments by PCR

Attempts were made to amplify marine-specific DNA fragments (F-1, II, III, and IV) by PCR with specific primers using the ten DNA samples from the North Pacific common minke whales. Fragment-I (F-I) was successfully amplified from all ten DNA samples (also see 2004b). Its nucleotide sequence was identical to F-I from *Brucella* spp. strain B2/94 from Atlantic seal. No DNA fragment could be amplified by PCR for F-II, III or IV. These findings indicate that the *Brucella* spp. from North Pacific common minke whales is more closely related to *Brucella* spp. from North Atlantic seal strains than one from North Atlantic cetaceans.

Table 1

Brucella-specific serum antibody from common minke whales collected under JARPN II 2000.

		Sub-area 7	Sub-area 9	Total (Sub-areas 7+9)
Male	Mature	25% (6-57)*, *n*=12	53% (27-79), *n*=15	41% (22-61), *n*=27
	Immature	57% (18-90), *n*=7	0% (0-98), *n*=1	50% (16-84), *n*=8
	Total	37% (16-62), *n*=19	50% (25-75), *n*=16	43% (26-61), *n*=35
Female	Mature	0% (0-60), *n*=4	nt	0% (0-60), *n*=4
	Immature	0% (0-98), *n*=1	nt	0% (0-98), *n*=1
	Total	0% (0-52), *n*=5	nt	0% (0-52), *n*=5
Total	Mature	19% (4-46), *n*=16	53% (27-79), *n*=15	35% (19-55), *n*=31
(Male+Female)	Immature	50% (16-84), *n*=8	0% (0-98), *n*=1	44% (14-79), *n*=9
	Total	29% (13-51), *n*=24	50% (25-75), *n*=16	38% (23-54), *n*=40

Males with seminiferous tubules over 100μm diameter or spermatid in the tubules were determined as sexually mature.
Sexual maturity for females was determined by the presence of at least one corpus luteum or albicans in either ovary.
* = % *Brucella* specific antibody prevalence (95% CI); nt = no sample.

Phylogenetic analysis of omp2 *from the whale samples*

Omp2 of *Brucella* was amplified by PCR in the ten North Pacific common minke whale samples. The nucleotide sequences showed that *Brucella* spp. from Pacific common minke whales had *omp2a* and *omp2b* (Gene Accession No.AB126348) (Ohishi *et al*., 2004b). The *omp2a* nucleotide sequence was identical to those of *Brucella* spp. strain B2/94 and strain F5/99 from an Atlantic seal and a Pacific bottlenose dolphin. The *omp2b* nucleotide sequence was identical to that of strain F5/99 (Cloeckaert *et al*., 2001; McDonald *et al*., 2006). Phylogenetic analyses using neighbour-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods were conducted (Ohishi *et al*., 2004a; 2004b). Sequence data from representative terrestrial strains (*B. melitensis*, *B. suis*, *B. ovis,* and *B. canis*), North Atlantic marine strains (porpoise B1/94 strain,

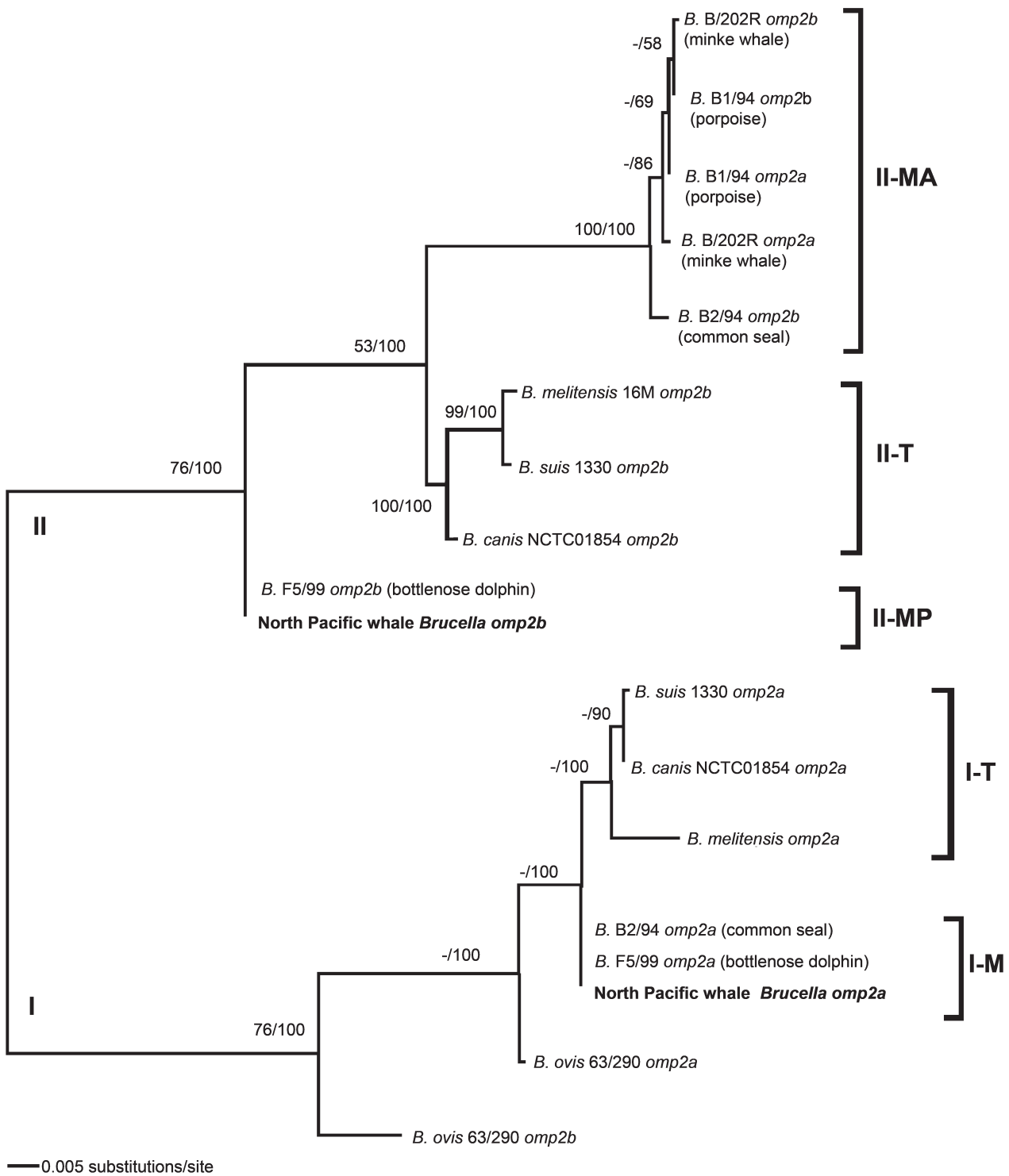

Fig. 1. Phylogenetic tree constructed by Maximum Likelihood (ML) method based on *Brucella omp2* gene sequences. Each number indicates the bootstrap probabilities (neighbour joining/maximum parsimony, respectively). The *Brucella* strains used in the analysis and their *GenBank* accession numbers are as follows: *B. melitensis* 16M (AE009568 AE009569); *B. suis* 1330 (AE014371); *B. ovis* 63/290 (U26442); *B. canis* NCTC01854 (U26439); porpoise strain B1/94 (AF300816, AF300817); Atlantic minke whale B/202R (AF027601); common seal B2/94 (AF300818, AF300819); Pacific bottlenose dolphin F5/99 (DQ865282, DQ865283); and North Pacific common minke whale M13 (AB126348). *B. ovis* exceptionally has two *omp2a* genes.

Atlantic minke whale B/202R strain, common seal B2/94 strain) and North Pacific strains (bottlenose dolphin strain F5/99, common minke whale strain M013/00), were also used. ML-based phylogeny of the *omp2* gene sequences from *Brucella* strains, is shown in Fig.1. The gene sequences fall into two distinct groups, I and II. In group I, *omp2a* genes of terrestrial *Brucella* strains formed a sub-clade (I-T), whereas *omp2a* genes from Atlantic seal and Pacific whale strains formed another sub-clade (I-M) that was independent of the I-T. In group II, three sub-clades were seen. All *omp2a* and *omp2b* genes from Atlantic cetacean strains belonged to one sub-clade (II-MA). Terrestrial strain *omp2b* genes formed another sub-clade (II-T). The Pacific whale *omp2b* belonged to an independent sub-clade (II-MP), which seemed to diverge basally within the radiation of clade II (Fig. 1). These phylogenetic analyses indicate that the *Brucella* spp. from Pacific whales is more closely related to *Brucella* spp. from Atlantic seals and terrestrial *Brucella* strains rather than to *Brucella* spp. from Atlantic cetaceans.

Serological examination of ships crew and researchers for the *Brucella* specific antibody

No clinical signs attributable to brucellosis were found in crews and researchers contacting the whales during whaling research activities. It is important to continue the monitoring. No positive reaction was observed for *Brucella* specific antibody in the serum samples collected from the 51

Table 2

Serologic study on *Brucella* spp. in marine mammals.

Ocean	Animal species (scientific name)	Positive rate (95% CI)	Reference
(a) Cetaceans			
Northern Hemisphere			
Eastern Atlantic (North Sea)	Porpoise (*Phocoena phocoena*)	11-28% (1-54) *n*=18	Ross *et al.* (1996)
Eastern Atlantic (Scotland)	Porpoise (*Phocoena phocoena*)	9% (2-24), *n*=35	Foster *et al.* (1996)
Eastern Atlantic (Norwegian Sea)	Fin whale (*Balaenoptera physalus*)	11% (6-19), *n*=108	Tryland *et al.* (1999)
Eastern Atlantic (Norwegian Sea)	Sei whale (*Balaenoptera borealis*)	14% (6-27), *n*=49	Tryland *et al.* (1999)
Eastern Atlantic (Norwegian Sea)	Minke whale (*B. acutorostrata*)	13% (5-26), *n*=46	Tryland *et al.* (1999)
Eastern Atlantic (England, Wales)	Porpoise (*Phocoena phocoena*)	31% (17-49), *n*=35	Jepson *et al.* (1997)
Eastern Atlantic (England, Wales)	Common dolphin (*Delphinus delphis*)	31% (15-51), *n*=29	Jepson *et al.* (1997)
Eastern Atlantic (Scotland)	Porpoise (*Phocoena phocoena*)	33% (26-41), *n*=152	Foster *et al.* (2002)
Mediterranean	Striped dolphin (*Stenella coeruleoalba*)	13% (2-38), *n*=16	Van Bressem *et al.* (2001)
Arctic Ocean (Canada)	Beluga (*Delphinapterus leucas*)	6% (4-8), *n*=488	Nielsen *et al.* (2001)
Arctic Ocean (Canada)	Narwhal (*Monodon monoceros*)	7% (2-15), *n*=77	Nielsen *et al.* (2001)
Arctic Ocean	Long-finned pilot whale (*Globicephala melas*)	0% (0-18), *n*=19	Nielsen *et al.* (2001)
Western Pacific (Japan)	Common minke whale (*B. acutorostorata*)	38% (23-54), *n*=40	Ohihsi *et al.* (2003)
Western Pacific (Japan)	Bryde's whale (*Balaenoptera edeni*)	9% (3-22), *n*=43	Ohishi *et al.* (2003)
Southern Hemisphere			
Antarctic	Antarctic minke whale (*Balaenoptera bonarensis*)	0% (0-4), *n*=104	Ohishi *et al.* (2003)
Western Pacific (Solomon Islands)	Bottlenose dolphin (*Turisops truncatus*)	53-69% (40-81), *n*=58	Tachibana *et al.* (2006)
Eastern Pacific (Peru)	Dusky dolphin (*Lagenorhynchus obscurus*)	78% (58-91), *n*=27	Van Bressem *et al.* (2001)
Eastern Pacific (Peru)	Burmeister's porpoise (*Phocoena spinpinnis*)	25% (9-49), *n*=20	Van Bressem *et al.* (2001)
(b) Pinnipeds			
Northern Hemisphere			
Eastern Atlantic (North Sea)	Common seal (*Phoca vitulina*)	18-49% (12-58), *n*=140	Ross *et al.* (1996)
Eastern Atlantic (North Sea)	Gray seal (*Halichoerus grypus*)	13-32% (4-51), *n*=31	Ross *et al.* (1996)
Eastern Atlantic (Scotland)	Gray seal (*Halichoerus grypus*)	6% (0-31), *n*=16	Foster *et al.* (1996)
Eastern Atlantic (Scotland)	Common seal (*Phoca vitulina*)	14% (4-33), *n*=28	Foster *et al.* (1996)
Eastern Atlantic (Barents Sea)	Harp seal (*Phoca groenlandica*)	2% (1-3), *n*=811	Tryland *et al.* (1999)
Eastern Atlantic (Barents Sea)	Hooded seal (*Cystophora cistata*)	35% (27-44), *n*=137	Tryland *et al.* (1999)
Eastern Atlantic (Barents Sea)	Bearded seal (*Erignathus barbatus*)	0% (0-21), *n*=16	Tryland *et al.* (1999)
Eastern Atlantic (Barents Sea)	Ringed seal (*Phoca hispida*)	10% (3-22), *n*=49	Tryland *et al.* (1999)
Eastern Atlantic (England, Wales)	Gray seal (*Halichoerus grypus*)	10% (4-20), *n*=62	Jepson *et al.* (1997)
Eastern Atlantic (England, Wales)	Common seal (*Phoca vitulina*)	8% (0-40), *n*=12	Jepson *et al.* (1997)
Eastern Atlantic (Scotland)	Common seal (*Phoca vitulina*)	49% (43-55), *n*=300	Foster *et al.* (2002)
Arctic Ocean (Greenland Sea)	Hooded seal (*Cystophora cistata*)	29% (15-51), *n*=29	Tryland *et al.* (2005)
Arctic Ocean (Canada)	Gray seal (*Halichoerus grypus*)	4% (2-7), *n*=255	Nielsen *et al.* (2001)
Arctic Ocean (Canada)	Common seal (*Phoca vitulina*)	13% (8-19), *n*=163	Nielsen *et al.* (2001)
Arctic Ocean (Canada)	Hooded seal (*Cystophora cistata*)	5% (2-9), *n*=204	Nielsen *et al.* (2001)
Arctic Ocean (Canada)	Harp seal (*Phoca groenlandica*)	2% (1-3), *n*=453	Nielsen *et al.* (2001)
Arctic Ocean (Canada)	Ringed seal (*Phoca hispida*)	1% (0-2), *n*=628	Nielsen *et al.* (2001)
Arctic Ocean (Canada)	Walrus (*Odobenus rosmarus*)	7% (2-16), *n*=70	Nielsen *et al.* (2001)
Eastern Pacific (Alaska)	Walrus (*Odobenus rosmarus*)	0% (0-9), *n*=40	Calle *et al.* (2002)
Eastern Pacific (Alaska)	Steller sea lions (*Eumetopias jubatus*)	1% (0-3), *n*=197	Burek *et al.* (2005)
Eastern Pacific (Alaska)	Common seal (*Phoca vitulina*)	46% (36-56), *n*=100	Zarnke *et al.* (2006)
Central Pacific	Hawaiian monk seal (*Monachus schaunsland*)	12-17% (7-24), *n*=164	Nielsen *et al.* (2005)
Central Pacific	Hawaiian monk seal (*Monachus schaunsland*)	0% (0-2) *n*=191	Aguirre *et al.* (2007)
Russia (Baikal Lake)	Baikal seal (*Phoca sibirica*)	0% (0-8), *n*=45	Ross *et al.* (1996)
South Hemisphere			
Antarctic	Antarctic fur seal (*Arctocephalus gazella*)	31% (11-59), *n*=16	Retamal *et al.* (2000)
Western Pacific (Australia)	Australian sea lion (*Neophoca cinerea*)	75% (43-95), *n*=12	Dawson (2005)
Western Pacific (New Zealand)	New Zealand fur seal (*Arctocephalus forsteri*)	0% (0-4), *n*=101	Mackereth *et al.* (2005)

All serologic data referred here from studies examining over 10 individuals from each species. Some data have multiple positive rates due to different assay systems used.

persons who participated in JARPN II in 2000 and from the 103 persons in JARPN II in 2003 by agglutination test using *B. abortus* antigens (Ohishi *et al*., 2004a; Ohishi *et al*., 2003).

DISCUSSION

A serological survey of the *Brucella* specific antibody in whales inhabiting the western North Pacific showed that exposure to *Brucella* spp. occurred in the whales examined from the western North Pacific. In particular, *Brucella* spp. seems to be prevalent in common minke whales. Although a systematic survey for the *Brucella* specific antibody was not conducted in small toothed whales on the coast of Japan, a specific *Brucella* serum antibody has also been found in two pygmy sperm whales (*Kogia breviceps*) stranded on the Pacific coast of Japan in 2001 and 2003 (Ohishi *et al*., 2007). *Brucella* spp. exposure seems to occur in other whale species in the western Pacific.

Testes with caseation and mineralisation were observed in 37% (13/35) of male common minke whales (Ohishi *et al*., 2003). The lesions were pathologically similar to ones induced by *Brucella* infection in terrestrial animals, whereas similar lesions have not been reported in infected marine mammals in other oceans (Foster *et al*., 2002). These lesions were observed only in mature males, despite the presence of the *Brucella* specific antibody in both mature and immature whales as shown in Table 1. To understand the formation and significance of these lesions, more intensive study is necessary. It is unknown how much *Brucella* spp. infection could impact aspects of breeding such as fertility, late term abortion and neonatal survival in these whales.

In contrast to common minke whales inhabiting the western North Pacific, Antarctic minke whales were all found to be negative for *Brucella* specific serum antibodies. Additionally, no abnormal gonads were found. These results indicate that Antarctic minke whales might not be exposed to *Brucella* spp., although the possibility that infection of less pathogenic *Brucella* spp. occurs at low prevalence cannot be excluded. This is congruent with the studies that minke whales in the Northern and Southern Hemispheres migrate exclusively within each hemisphere. Recent seroepidemiological studies on *Brucella* spp. in a variety of marine mammals, are summarised in Table 2. In the Southern Hemisphere, *Brucella* infection has been reported from small toothed whales near Solomon Island (9°7'S, 160°10.6'E), and off Peru (Tachibana *et al*., 2006; Van Bressem *et al*., 2001), although data from southern oceans is very limited. Continuous surveillance is needed to investigate whether Antarctic minke whales continue to be seronegative for *Brucella* spp. These epidemiological data give an insight into possible interactions between whales in the ocean.

Molecular analyses showed *Brucella* spp. from North Pacific common minke whales has an insertion of IS*711* downstream of *bp26*, a specific marker for the marine strain (Ohishi *et al*., 2004b). This indicates that the *Brucella* spp. from Pacific common minke whales is closely related to marine strains identified thus far, although the whales inhabiting the Pacific and Atlantic Oceans have no direct contact at the present. *Brucella* spp. in marine mammals does not seem to have been recently introduced from terrestrial animals based on analysis of currently known strains. However, further surveys in a variety of animals is needed to obtain a conclusion for exchange of *Brucella* spp. in wild animals. Interestingly, *Brucella* spp. from Pacific minke whales has more similarity to strains from Atlantic seals. This shows that classification based on the marine mammal host species, *B. cetacea* and *B. pinnipedia* is not appropriate. *Omp2b* from Pacific whales branches early within the radiation of clade II, as shown in Fig. 1. This may indicate that *Brucella* spp. from Pacific minke whales is an archetype. Recent studies have shown *omp2* genes have chimeric molecular structures between *omp2a* and *omp2b* in each strain (Cloeckaert *et al*., 2001; Ohishi *et al*., 2005). The two *omp2b* genes found in Atlantic cetacean strains, may have been formed by gene conversion (Cloeckaert *et al*., 2001; Ohishi *et al*., 2005). In addition to *omp2* genes, other genes should be characterised to understand the phylogenetic relationship between *Brucella* strains.

The serum tests of crews and researchers participating in JARPN II were conducted independently, on two separate occasions, and results showed no sign of seroconversion to marine *Brucella* spp. in humans. The results were consistent with the fact that to date there have been no reports of brucellosis due to consumption of, or contact with marine mammals, although exposure to marine *Brucella* strains in the laboratory has been reported (Brew *et al*., 1999; Foster *et al*., 2002; Godfroid *et al*., 2005; Tryland *et al*., 1999). It is important to continue the careful monitoring of people working with marine mammals or working on *Brucella* in the laboratory.

Despite repeated trials using a variety of tissues from common minke whales, it was not possible to isolate *Brucella* spp. This result may be partially explained due to the limited bacteria antigens available in the lesions for the detection. However, to understand the characteristics of the Pacific whale *Brucella* spp., efforts should continue to isolate *Brucella* strains.

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