Brucella infection in whales in the western North Pacific and Antarctic: A review

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Key word: Diseases

ABSTRACT

Infection of *Brucella*, Gram-negative, pathogenic bacteria, has been reported in a variety of marine mammals in worldwide oceans. We have conducted serological and pathological studies on *Brucella* infection in the western North Pacific using whale samples collected in 2000 under the second phase of the Japanese Whale Research Program under Special Permit in the Western North Pacific (JARPNII). Serum samples from 40 common minke whales (*Balaenoptera acutorostrata*), 43 Bryde's whales (*Balaenoptera edeni*), and 4 sperm whales (*Physeter macrocephalus*) were assessed by agglutination test using *B. abortus*. *Brucella*-specific antibodies were detected in 38% of common minke whale samples. Low prevalence (9%) of the antibody was observed in Bryde's whale samples, whereas no antibody against *Brucella* was observed in the examined four sperm whales. We also investigated the serum samples from 104 Antarctic minke whales (*Balaenoptera bonaerensis*) collected under the Japanese Whale Research Program under Special Permit in the Antarctic (JARPA), and no antibodies were detected in the sera. We found abnormal gonads in 35% (13 males and one female) of 40 minke whales, having granular lesions with caseation and calcification. Similar lesions were also observed in the gonads of two Bryde's whales. Such gonad abnormalities were not observed in 440 Antarctic minke whales and five sperm whales. Histopathological studies showed that the lesions consisted of epithelioid cells, multinucleated giant cells and had infiltration of lymphocytes. These results suggested the followings, (i) *Brucella* infection occurred in the examined whale species in the western North Pacific, (ii) a relatively higher infection rate was observed in common minke whales, and (iii) *Brucella* infection does not seem to occur in the Antarctic minke whales. 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*. The gene structure of *omp2*, and specific PCR products for seal strains, showed similarity to Atlantic seal strains. Thus far, crews and researchers who have had frequent contact with whales, have no health complexities. No *Brucella*-specific antibody was detected in the sera from 51 persons examined in 2001, nor from 103 in 2003.

INTRODUCTION

Brucella, a genus of Gram-negative bacteria, is a causative agent of brucellosis, a worldwide zoonotic disease (Corbel and Brinley-Morgan 1984). Brucellosis has been studied in domesticated mammals, such as cattle, sheep, and pigs, and is known to cause reproductive disorders or abortions in infected animals. Six species are recognized within *Brucella* originated 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 Brinley-Morgan 1984). *Brucella* species may cause abortion, orchitis, epididymitis, arthritis, and/or spondylitis in these domesticated animals, and result in economic losses. *Brucella* has also been isolated from a variety of terrestrial wildlife mammal species such as bison, elk, buffalo, reindeer, caribou, 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 (Ross et al., 1994, Ewalt et al., 1994). Phenotypic and bacteriological characteristics of the marine strains differed from those of the six terrestrial species (Jahans et al., 1997, Clavareau et al., 1998). Molecular biological analysis has further supported that the marine strains are different from terrestrial ones (Clavareau et al., 1998, Cloeckaert et al., 2000, 2001, 2003). These studies have suggested that the *Brucella* marine strains were not recently introduced from terrestrial animals, and might have evolved with marine mammals.

Seroepidemiological analysis is useful in investigating the distribution and host range of *Brucella* in oceans. Much of the information currently known is from the North Hemisphere, especially from waters around Europe and USA. Data from the western Pacific and Southern Hemisphere is very limited.

In addition to these serologic investigations, molecular analysis is also important to identify the strains and to provide an information on the bacterial transmission and evolution. Here we review recent studies of serology, pathology and molecular biology of *Brucella* infection in baleen whales inhabiting the western North Pacific and Antarctic Oceans.

SEROLOGIC SURVEY IN WHALES

We conducted a serological study on *Brucella* infection in the western North Pacific using whale samples 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 (JARPNII). Serum samples from 40 common minke whales (*Balaenoptera acutorostrata*), 43 Bryde's whales (*Balaenoptera edeni*), and 4 sperm whales (*Physeter macrocephalus*) were assessed by agglutination test using *B. abortus*. *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 summarized in Table 1. Low prevalence (9%) of the antibody was observed in Bryde's whale samples, and no antibodies were detected in the examined four sperm whales. Serum samples from 104 Antarctic minke whales (*Balaenoptera bonaerensis*) obtained in 2000/2001 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 (i) *Brucella* infection occurred in the examined whale species in the western North Pacific in the 2000 survey, (ii) relatively higher infection rate was observed in the common minke whale, and (iii) *Brucella* infection does not occur in the Antarctic minke whale.

Table 1 Specific antibody against *Brucella* in serum samples from common minke whales collected under JARPNII2000.

		Sub-area 7	Sub-area 9	Total (Sub-areas 7+9)
Male	Mature	25%(3/12)	53%(8/15)	41%(11/27)
	Immature	57%(4/7)	0%(0/1)	50%(4/8)
	Total	37%(7/19)	50%(8/16)	43%(15/35)
Female	Mature	0%(0/4)	nt	0%(0/4)
	Immature	0%(0/1)	nt	0%(0/1)
	Total	0%(0/5)	nt	0%(0/5)
Total (Male + Female)	Mature	19%(3/16)	53%(8/15)	35%(11/31)
	Immature	50%(4/8)	0%(0/1)	44%(4/9)
	Total	29%(7/24)	50%(8/16)	38%(15/40)

Males with seminiferous tubles over 100um diameter or spermatid in the tubles were determined as

sexually mature. Sexual maturity for females was determined by the presence of at least one corpus luteum or albicans in both ovaries. nt; no sample.

HISTOPATHOLOGICAL STUDY OF THE ABNORMAL GONADS

Abnormal lesions were observed in the testes and uterine endometrium in 35% (13 males and one female) of common minke whales in the JARPNII2000 survey. The lesions were characterized as granular lesions with caseation and calcification (Ohishi et al., 2003). Similar lesions were found in epididymis and ovary in two mature Bryde's whales. Such gonad abnormalities 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, which are known to induce similar lesions in lung tissue. These results support the whales as being infected with *Brucella*. However, we did not observe any positive reactions with anti-*B. abortus* antibodies in these lesions by immunostaining.

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 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 marine isolates (Cloeckaert et al., 2003). Fragment I (F-I) is specific for seal *Brucella* isolates, whereas Fragments-II, III, and IV (F-II, III, and IV) are cetacean- *Brucella* -specific. On the other hand, 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* which has 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 subareas 7. 8, and 9 under JARPNII2000 and JARPNII2001. *Brucella* specific DNA fragments were detected by PCR with specific primers in ten among 22 samples (Ohishi et al., 2004a).

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., 2004a). This result suggests that *Brucella* from North Pacific common minke whales have a close relationship to *Brucella* isolates from marine mammals in the North Atlantic.

Detection of marine-specific DNA fragments by PCR

Marine-specific DNA fragments (PCR-I, II, III, and IV) were amplified by PCR with specific primers using the ten DNA samples from the North Pacific common minke whales. Fragment-I (F-I) was also amplified by the specific PCR from all ten samples (Ohishi et al., 2004a). Their nucleotide sequences were completely identical to F-I from the Atlantic seal strain B2/94. No DNA fragment could be amplified by PCR for fragment-II, III, or IV. These findings indicate that the *Brucella* from North Pacific common minke whales was more closely related to North Atlantic seal strains than to North Atlantic cetacean strains.

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 Pacific common minke whale *Brucella* had *omp2a* and *omp2b* (Gene Accession No.AB126348)(Ohishi et al., 2004a). The *omp2a* nucleotide sequence was identical to those of the Atlantic seal strain B2/94 and the Pacific bottlenose dolphin strain F5/99. The *omp2b* nucleotide

sequence was identical to that of the Pacific bottlenose dolphin strain F5/99 (Cloeckaert et al., 2001, McDonald et al., 2006)). Phylogenetic analyses using by neighbor-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*, and *B. canis*), North Atlantic marine strains (porpoise B1/94 strain, 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. 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 other sub-subclade (I-M). 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 diverged basally within the radiation of clade II. These phylogenetic analyses indicate that the *Brucella* from Pacific whales is more closely related to Atlantic seal strains and terrestrial *Brucella* strains rather than to Atlantic cetacean *Brucella* strains.

SEROLOGICAL EXAMINATION OF ANTIBODY AGAINST BRUCELLA OF CREWS AND RESEARCHERS

No health complexity has been observed in crews and researchers who had the potential to come into contact with whales during whaling research conducted thus far. We examined the specific antibody in the serum samples collected from the 51 persons who participated in JARPNII in 2000 and from the 103 persons in JARPNII in 2003 by agglutination test using *B. abortus* antigens (Ohishi et al., 2003, 2004b). No positive reaction was observed.

DISCUSSION

Serologic survey on *Brucella* infection in whales inhabiting the western North Pacific showed that *Brucella* infection occurred in the examined whales in the western North Pacific. In particular, *Brucella* seems to be prevalent in common minke whales. Although a systematic serologic survey on *Brucella* infection was not conducted in small toothed whales on the coast of Japan, we have additionally found a specific *Brucella* antibody in two pygmy sperm whales stranded on the Pacific coast of Japan in 2001 and 2003, respectively (Ohishi et al., 2007). *Brucella* infection in whales in the western Pacific seems to occur in a variety of whales as seen in other oceans.

Abnormal testes with caseation and calcification 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 of the presence of the *Brucella* specific antibody in both mature and immature whales as shown in Table 1. To understand the formation of these lesions, more study is necessary. It is unknown how much effect *Brucella* infection has on breeding in these whales.

In contrast to common minke whales inhabiting the western North Pacific, Antarctic minke whales were all negative for *Brucella* antibodies. Additionally, no abnormal gonads were found. These results indicated *Brucella* has not infected to the Antarctic minke whales. This is consistent with the ecology of minke whales in the Northern and Southern Hemispheres, the whales in each hemisphere migrate exclusively within each hemisphere, and are completely separated. In the Southern Hemisphere, *Brucella* infection has been reported from small toothed whales in Solomon Island (9° 7'S, 160° 10.6'E), and off Peru (Tachibana et al., 2005, Van Bressem et al., 2000), although data from southern oceans is very limited. Continuous surveillance is needed to investigate if Antarctic minke whales are always seronegative for *Brucella* or whether bacterial transmission occurs between whale species. Epidemiological data may give add important information on unknown cetacean biology such as population structure, distribution, migration, and feeding.

Molecular analyses showed *Brucella* in the North Pacific common minke whale *Brucella* has an insertion of IS*711* downstream of *bp26*, a specific marker for marine strain (Ohishi et al., 2004a). This indicates that the Pacific common minke whale *Brucella* is closely related to marine strains identified

thus far, although the whales inhabiting in the Pacific and Atlantic Oceans have no direct contact at the present. Thus, *Brucella* in marine mammals does not seem to have been recently introduced from terrestrial animals. Interestingly, Pacific whale *Brucella* has more similarity to Atlantic seal strains rather than to Atlantic cetacean strains. *omp2b* from Pacific whales branches early within the radiation of clade II. This may indicate that Pacific whale *Brucella* 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., 2006). Two *omp2b* genes found in Atlantic cetacean strains, may have been formed by gene conversion (Cloeckaert et al., 2001, Ohishi et al., 2006).

The serum tests of crews and researchers attended on JARPNII were conducted at two separate times independently, and results showed no sign of transmission of marine *Brucella* infection to 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 (Tryland et al., 1999, Foster et al., 2002, Godfroid et al., 2005). It is important to continue the monitoring carefully.

Despite repeated trials using a variety of tissues from common minke whales, we have failed to isolate *Brucella*. This result may be partially explained due to the limited amount of bacteria antigens in whale tissues. However, to understand characteristics of Pacific whale *Brucella*, continuous efforts must be taken to isolate *Brucella* strains. Further research will provide insights on *Brucella* infection in marine mammals at a global level, as well as on transmission and evolution of this bacteria genus.

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