



NOTE

Wildlife Science

## Serologic survey of *Brucella* infection in cetaceans inhabiting along the coast of Japan

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**ABSTRACT.** A serologic investigation of *Brucella* infection was performed in 7 species of cetaceans inhabiting along the coast of Japan. A total of 32 serum samples were examined by enzyme-linked immunosorbent assay (ELISA) using *Brucella abortus* and *B. canis* antigens. One serum sample from five melon-headed whales (*Peponocephala electra*) was positive for *B. abortus*. No serum sample showed positive for *B. canis*. The ELISA-positive melon-headed whale serum demonstrated a strong band appearance only against *B. abortus* antigens in Western blot analysis. Many detected bands were discrete, while some of them had a smeared appearance. The present results indicate that *Brucella* infection occurred in melon-headed whale population and the bacterial antigenicity is more similar to that of *B. abortus* than *B. canis*.

**KEY WORDS:** antibody, *Brucella*, cetacean, whale

*Brucella*, Gram-negative intracellular bacteria, infect variety of mammals and induce reproductive disorders including abortion [5]. In marine mammals, *B. ceti*, which is preferentially associated with cetaceans, and *B. pinnipedialis*, which is preferentially associated with pinnipeds, have been identified [7]. Compared to terrestrial *Brucella* species, these marine *Brucella* species seem to induce less pathological changes. However, in *B. ceti* infection, there have been substantial number of pathological reports including abnormalities in reproductive organs or nervous system [8, 9]. The abortion-inducing potential of *B. ceti* has been shown in captive and stranded dolphins and porpoises [6, 10, 13].

Extensive microbiological and serologic investigations have been performed on animals mainly from European and American waters, and revealed that many marine mammal species were infected with *Brucella* [8, 9]. In the western North Pacific, long-term and large-scale epidemiological surveys have shown that *Brucella* infection occurred in three species of baleen whales; common minke whales (*Balaenoptera acutorostrata*), Bryde's whales (*B. brydei*), and sei whales (*B. borealis*), and sperm whales (*Physeter macrocephalus*) [17, 20]. Especially, high prevalence of anti-*Brucella* antibodies and granulomatous testes were observed in common minke whales [17, 20]. Serologic evidence of *Brucella* has been found in two pygmy sperm whales (*Kogia breviceps*) stranded on the Japanese coast of the Pacific Ocean [18]. However, the available information is still limited in the Asian waters. A serologic survey was conducted to investigate the status of *Brucella* infection around Japan.

The serum samples used in the present study were summarized in Table 1 and Fig. 1. The blood samples were collected from cetaceans stranded and incidentally caught by a setnet, according to the guideline of the Japanese Fisheries Agency. The blood

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**Table 1.** Prevalence of serum antibodies to *Brucella* in cetaceans inhabiting the coast of Japan

Species	Year	Location				Positivity rate <sup>b)</sup>	
		Prefecture	City	Town	Map site <sup>a)</sup>	<i>B. abortus</i>	<i>B. canis</i>
Dall's porpoises ( <i>Phocoenoides dalli</i> )	2001			Off Iwate Prefecture	1	0/20	0/20
Melon-headed whales ( <i>Peponocephala electra</i> )	2001–2002	Ibaraki	Kamisu	Hasaki	2	1/5	0/5
Harbor porpoise ( <i>Phocoena phocoena</i> )	2017	Hokkaido	Hakodate	Usujiri	3	0/3	0/3
Pacific white-sided dolphin ( <i>Lagenorhynchus obliquidens</i> )	2017	Hokkaido	Hakodate	Shiokubi	4	0/1	0/1
Minke whale ( <i>Balaenoptera acutorostrata</i> )	2001	Kanagawa	Fujisawa	Enoshima	5	0/1	0/1
Hubbs' beaked whale ( <i>Mesoplodon carlhubbsi</i> )	2005	Kanagawa	Ninomiya	Yamanishi	6	0/1	0/1
Dwarf sperm whale ( <i>Kogia sima</i> )	2007	Niigata	Niigata	Matsuhaminato	7	0/1	0/1

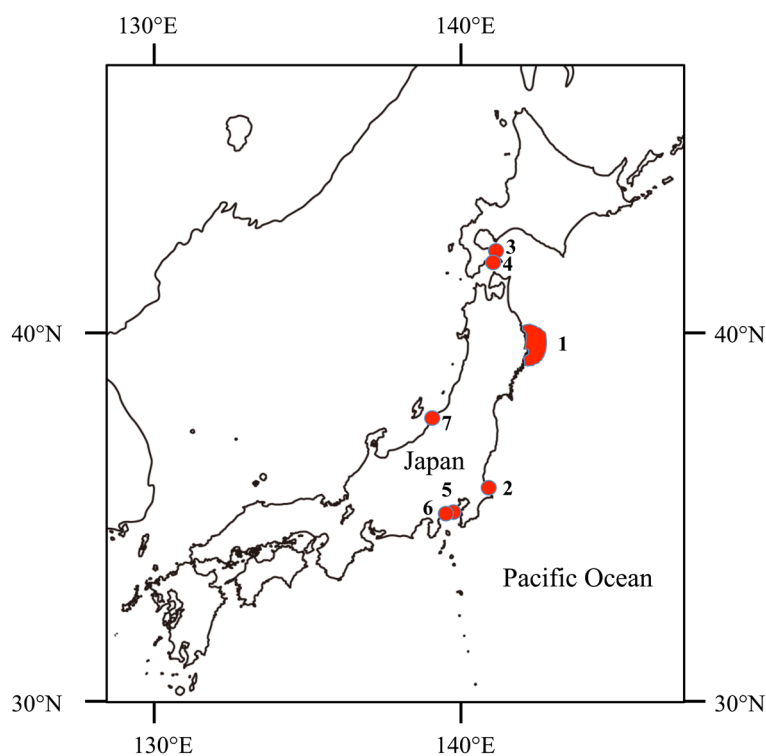
a) Numbers indicate the site in Fig. 1. b) Positive individuals /examined individuals. Absorbance greater than 0.2 at 405 nm is regarded as positive.

samples of Dall's porpoises (*Phocoenoides dalli*) captured by a commercial fishery were also collected. Three harbor porpoises (*Phocoena phocoena*) rescued from a setnet were kept according to the guidelines for regulations on animal experimentation of Hokkaido University and were released after a medical care.

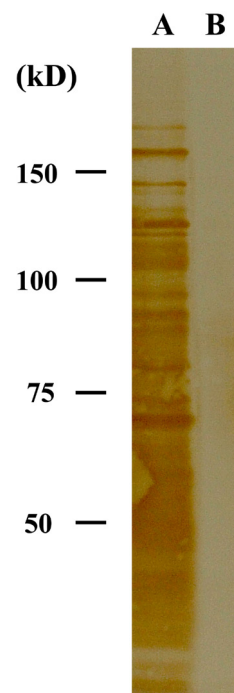
Anti-*Brucella* serum antibody was detected in the enzyme-linked immunosorbent assay (ELISA) and in the Western blot analysis according to the protocol described previously [1, 17]. Briefly, commercially available inactivated *B. abortus* strain 125 (Kaketsuken Co., Kumamoto, Japan) and *B. canis* strain QE-13B (Kitasato Institute Co., Tokyo, Japan) were solubilized and used as antigens in both methods. The sera diluted to 1:100 and horseradish peroxidase-conjugated Protein A/G (Thermo Fisher Scientific Inc., Waltham, MA, USA) diluted to 1:5,000, were used for the primary antibody and for its detection in both methods. In the ELISA, the absorbance value higher than 0.2 at 405 nm, was regarded as positive [1, 16].

One serum sample from a female melon-headed whale (*Peponocephala electra*) (ID: PE57) showed a positive-value (OD=0.34) only for *B. abortus* antigens. However, none of the serum samples showed positive to *B. canis* antigens (Table 1). In Western blot analysis, the ELISA-positive melon-headed whale serum showed a strong response only to *B. abortus* (Fig. 2). Many bands, including the smeary bands, were detected against *B. abortus*, while no band against *B. canis* was detected at this serum concentration (Fig. 2).

The present results indicated that the *Brucella* infection occurred in melon-headed whale population, and the *Brucella* strain



**Fig. 1.** Sampling sites. Numbers in the map correspond to those of locations in Table 1.



**Fig. 2.** Western blot analysis of the serum from an ELISA-positive melon-headed whale (ID: PE57) against *Brucella abortus* and *B. canis* antigens. A, *B. abortus* antigen. B, *B. canis* antigen.

was antigenetically closer to *B. abortus* than *B. canis*. The appearance of the smeared bands suggests that the infected *Brucella* are smooth (S) colony type of bacteria containing large O-type polysaccharide in the outer membrane as well as *B. abortus* [23]. These serum responses are similar to those of three species of baleen whales and sperm whales in the western North Pacific, and beluga whales in the Anadyr Firth, Russia [16, 17]. This suggests that S colony type of *Brucella* are prevalent in at least several cetacean species in the western North Pacific.

Melon-headed whales are distributed in the tropical and subtropical waters, and several mass stranding events of melon-headed whales have been recorded on multiple coasts of Japan [2, 12]. In Aoshima town in Miyazaki prefecture, the mass stranding with 135 individuals was recorded for the first time in 1982 [14, 15]. More recently, 171 whales at Tanegashima Island in Kagoshima prefecture and 85 whales at Hasaki town in Ibaraki prefecture, stranded in 2001 and 2002, respectively [4]. Comparison of these animals in term of animals' body size suggests that the melon-headed whales found in various parts of Japan are composed of several geographically different regional populations [4]. It would be interesting to compare the antibody positivity among these regional populations of the species, because maternal transmission is considered to be a major route of *Brucella* in cetaceans [17]. We have previously reported the serologic evidence of *Brucella* infection in two pygmy sperm whales which also inhabit in the tropical and subtropical waters [18, 22]. Because the investigation on *Brucella* infection has been rarely conducted in these waters, it is important to accumulate the epidemiological information through surveillance in Asian waters.

No specific antibodies were found in the examined 20 Dall's porpoises. It is a contrast to common minke whales inhabiting the western North Pacific with a high prevalence of the antibodies (9–38% during 1994–2010), though the habitats of the two species are partially overlapped [3, 17, 19]. Similarly, serologically *Brucella* positive or negative whale species inhabit in the western South Hemisphere. Bottlenose dolphins (*Tursiops truncatus*) inhabiting around the Solomon islands have shown high prevalence of anti-*Brucella* antibodies in their sera [21]. However, Antarctic minke whales (*Balaenoptera bonarensis*) migrating the western South Pacific seem to be *Brucella*-free, because specific antibodies have not been detected in the examined 739 serum samples [11, 17]. These facts may indicate that environmental transmission of *Brucella* is less likely to occur in cetaceans. The present serum samples from Dall's porpoises were collected off Iwate prefecture in 2001, before being seriously damaged by the 2011 Great East Japan Earthquake. The present data would provide a serologic starting line for ongoing or future researches of the species after the disaster. Further studies not only by serology but also by bacterial isolation should be performed in the future. The microbiological and molecular biological characterizations are necessary for understanding the distribution of *Brucella* and its impact on cetaceans in Asian waters.

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