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Antibodies to *Brucella* spp. in Pacific Bottlenose Dolphins from the Solomon Islands

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ABSTRACT: *Brucella* spp. have been recently isolated from a variety of marine mammals. Serum samples from 58 Pacific bottlenose dolphins (*Tursipa aduncus*) from the Solomon Islands were tested for antibodies to *Brucella* spp. by the tube agglutination test (TAT), enzymed-linked immunosorbent assay (ELISA), and immunoblotting. Anti-*Brucella* spp. antibodies were detected by TAT and ELISA in 31 and 40 of 58 samples, respectively. These results suggest that Pacific bottlenose dolphins from the Solomon Islands are infected with *Brucella* spp. or a *Brucella*-like organism.

Key words: Bottlenose dolphin, *Brucella*, Solomon Islands.

Brucellosis is a serious debilitating disease in humans and an important cause of abortion and sterility in domestic animals. *Brucella* spp. are facultative, gram-negative, intracellular bacteria and the etiologic agent of brucellosis, a widely distributed zoonosis (Ko and Splitter, 2003). The establishment of chronic infection depends on the ability of brucellae to survive within phagocytes (Harmon et al., 1988). The host range for *Brucella* spp. has recently expanded to include marine mammals, and anti-*Brucella* antibodies have been detected in cetaceans and pinnipeds around Europe and North and South America as well as the Arctic Sea (Nielsen et al., 1996; Ross et al., 1996; Garner et al., 1997; Jepson et al., 1997; Tryland et al., 1999; Forbes et al., 2000; Retamal et al., 2000; Van Bressem et al., 2001; Ohishi et al., 2003).

In this study we tested Pacific bottlenose dolphins (*Tursiops aduncus*) from the Solomon Islands for antibodies to *Brucella* spp. Serum samples were obtained from 58 Pacific bottlenose dolphins captured in 2003 at Gavutu Island, Solomon Islands (9°7′S, 160°10.6′E). Blood samples were taken with the approval of the Solomon Islands government and with Animal Care and Use approval in the Solomon Islands (Omata et al., 2005). All dolphins were captured with nets as described (Geraci and Lounsbury, 1993); all animals remained alive and were later released or used in a dolphin swim program.

The tube agglutination test (TAT) was used for the detection of anti-*Brucella* antibodies. An antibody titer of ≥40 is regarded as positive (100 international units/ml) in testing of domestic animals. Serum samples were also tested by ELISA and immunoblotting as described previously (Erdenebaatar et al., 2003). For ELISA, the positive threshold value was set as the lowest absorbance value (OD_{492}=0.4) observed for serum samples testing positive by TAT at dilutions of 1:40. An absorbance of higher than this at a dilution ratio 1:40 was regarded as positive, and all samples were diluted to endpoint. Antibodies to *Brucella* were detected by TAT in 31 of 58 serum samples and by ELISA in 40 of 58 serum samples that tested positive. ELISA absorbance values for 18 of the TAT-negative samples ranged between 0.05 and 0.40 for all dilutions. In the remaining nine TAT-negative samples, absorbance ≥0.4 were observed. We believe that this relates to increased sensitivity associated with the ELISA test. Antibody titers as determined by ELISA for the 40 antibody positive samples were distributed as fol-

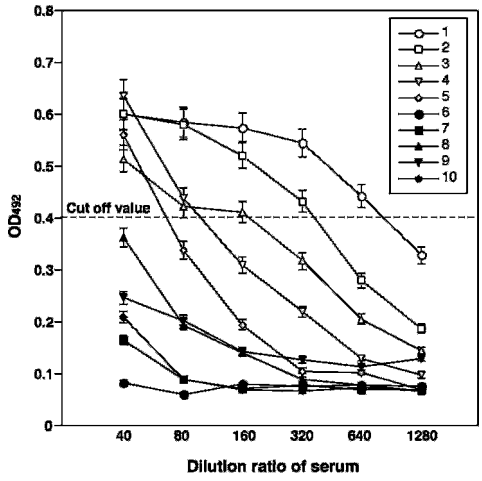

FIGURE 1. Measurement of anti-*Brucella* antibodies in Pacific bottlenose dolphin sera by ELISA. The lowest absorbance value of serum samples testing positive by TAT at dilutions of 1 : 40 was tentatively taken as the cutoff value (OD_{492}=0.4). Data are shown for 10 of the 58 serum samples. Data are the averages and standard deviations of triplicate wells.

lows: 40 (11 samples), 80 (13 samples), 160 (11 samples), 320 (3 samples), and 640 (2 samples; Fig. 1). By immunoblotting, all 40 samples were found to have antibody binding to *B. abortus* antigens, but not to *B. canis* antigens, at dilution of 1:100.

Our results indicate that 53% of the dolphins had anti-*Brucella* antibodies; these were detected using three assays: TAT, ELISA, and immunoblotting. Therefore, there is a possibility that dolphins in the Solomon Islands area are infected with *Brucella* spp. or a *Brucella*-like organism. For serodiagnosis of acute and recent infections with *Brucella* spp. and *Yersinia enterocolitica* O9, the commonly used agglutination assay is seriously impaired by the well-documented serologic cross-reactivity between these bacteria (Diaz-Aparicio et al., 1993). We have previously reported that ELISA results based on antigens extracted from *B. abortus* with *n*-lauroylsarcosine differentiated natural *Brucella*-infected animals from *Y. enterocolitica* O9–infected animals (Erdenebaatar et al., 2003). Serological cross-reactions between *Brucella* species and species in other genera, however, have been reported, including cross-reactions with *Pasteurella* spp., *Salmonella enterica* serotype Urbana or Pullorum, *Francisella tularensis*, and *Escherichia coli* O157 (Corbel, 1985). Although our results strongly suggest previous infection with *Brucella* spp., the possibility of cross-reactions cannot be totally discounted.

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