

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

Authors: Tachibana, Masato, Watanabe, Kenta, Kim, Suk, Omata, Yoshitaka, Murata, Koichi, et al.

Source: Journal of Wildlife Diseases, 42(2) : 412-414

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-42.2.412>

BioOne Complete (complete.BioOne.org) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at www.bioone.org/terms-of-use.

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

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

Masato Tachibana,¹ Kenta Watanabe,¹ Suk Kim,¹ Yoshitaka Omata,² Koichi Murata,³ Ted Hammond,⁴ and Masahisa Watarai^{1,5} ¹ Department of Applied Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan; ² Department of Basic Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan; ³ Department of Wildlife Science, College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 252-8510, Japan; ⁴ Hammond Consultants Inc., 1881 Rouge River Circle, Ventura, California 93004, USA; ⁵ Corresponding author (email: watarai@obihiro.ac.jp)

ABSTRACT: *Brucella* spp. have been recently isolated from a variety of marine mammals. Serum samples from 58 Pacific bottlenose dolphins (*Tursiops aduncus*) from the Solomon Islands were tested for antibodies to *Brucella* spp. by the tube agglutination test (TAT), enzyme-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 Bresseem 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-

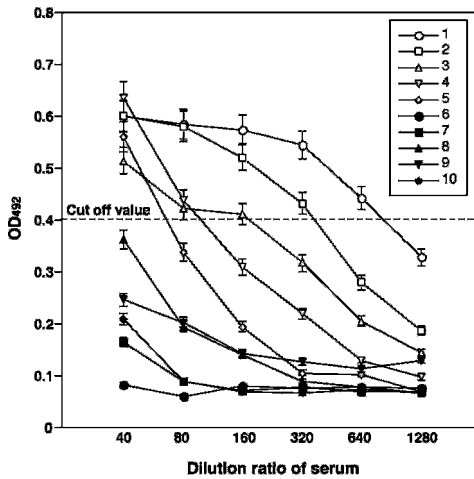


FIGURE 1. Measurement of anti-*Brucella* antibodies in Pacific bottlenose dolphin sera by ELISA. The lowest absorbance value of serum samples testing positive by TAT at dilutions of 1 : 40 was tentatively taken as the cutoff value ($OD_{492}=0.4$). Data are shown for 10 of the 58 serum samples. Data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

We thank Dr. Alexander Cox for critical reading of the manuscript. This work was supported, in part, by grants from Scientific Research (16017207 and 16790250), the Japan Society for the Promotion of Science, and the Inamori Foundation.

LITERATURE CITED

- CORBEL, J. M. 1985. Recent advances in the study of *Brucella* antigens and their serological cross-reactions. *Veterinary Bulletin* 55: 927-942.
- DIAZ-APARICIO, E., V. ARAGON, C. MARIN, B. ALONSO, M. FONT, E. MORENO, S. PEREZ-ORTIZ, J. M. BLASCO, R. DIAZ, AND I. MARIYON. 1993. Comparative analysis of *Brucella* serotype A and M and *Yersinia enterocolitica* O9 polysaccharides for serological diagnosis of brucellosis in cattle, sheep, and goats. *Journal of Clinical Microbiology* 31: 3136-3141.
- ERDENEBAATAR, J., B. BAYARSAIKHAN, M. WATARAI, S. MAKINO, AND T. SHIRAHATA. 2003. Enzyme-linked immunosorbent assay to differentiate the antibody responses of animals infected with *Brucella* species from those of animals infected with *Yersinia enterocolitica* O9. *Clinical and Diagnostic Laboratory Immunology* 4: 710-714.
- FORBES, L. B., O. NIELSEN, L. MEASURES, AND D. R. EWALT. 2000. Brucellosis in ringed seals and harp seals from Canada. *Journal of Wildlife Diseases* 36: 595-598.
- GARNER, M. M., D. M. LAMBOURN, S. J. JEFFRIES, P. B. HALL, J. C. RHYAN, D. R. EWALT, L. M. POLZIN, AND N. F. CHEVILLE. 1997. Evidence of *Brucella* infection in Parafilaroides lungworms in a Pacific harbor seal (*Phocavitulina richardsi*). *Journal of Veterinary Diagnostic Investigation* 9: 298-303.
- GERACI, J. R., AND V. J. LOUNSBURY. 1993. Marine mammals ashore a field guide for strandings. Texas A&M Sea Grant publication, Galveston, Texas, 51 pp.
- HARMON, B. G., L. G. ADAMS, AND M. FREY. 1988. Survival of rough and smooth strains of *Brucella abortus* in bovine mammary gland macrophages. *American Journal of Veterinary Research* 49: 1092-1097.

- JEPSON, P. D., S. BREW, A. P. MACMILLAN, J. R. BAKER, J. BARNETT, J. K. KIRKWOOD, T. KUIKEN, I. R. ROBINSON, AND V. R. SIMPSON. 1997. Antibodies to *Brucella* in marine mammals around the coast of England and Wales. *Veterinary Record* 141: 513–515.
- KO, J., AND G. A. SPLITTER. 2003. Molecular host-pathogen interaction in brucellosis: Current understanding and future approaches to vaccine development for mice and humans. *Clinical Microbiology Reviews* 12: 405–428.
- NIELSEN, O., K. NIELSEN, AND R. E. A. STEWART. 1996. Serologic evidence of *Brucella* spp. exposure in Atlantic walrus (*Odobenus rosmarus rosmarus*) and ringed seals (*Phoca hispida*) of Arctic Canada. *Arctic* 49: 383–386.
- OHISHI, K., R. ZENITANI, T. BANDO, Y. GOTO, K. UCHIDA, T. MARUYAMA, S. YAMAMOTO, N. MIYAZAKI, AND Y. FUJISE. 2003. Pathological and serological evidence of *Brucella*-infection in baleen whales (*Mysticeti*) in the western North Pacific. *Comparative Immunology, Microbiology and Infectious Diseases* 26: 125–136.
- OMATA, Y., T. HAMMOND, K. ITOH, AND K. MURATA. 2005. Antibodies against *Toxoplasma gondii* in the pacific bottlenose dolphin (*Tursiops aduncus*) from Solomon Islands. *Journal of Parasitology* 91: 965–967.
- RETAMAL, P., O. BLANK, P. ABALOS, AND D. TORRES. 2000. Detection of anti-*Brucella* antibodies in pinnipeds from the Antarctic territory. *Veterinary Record* 146: 166–167.
- ROSS, H. M., K. L. JAHANS, A. P. MACMILLAN, R. J. REID, P. M. THOMPSON, AND G. FOSTER. 1996. *Brucella* species infection in North Sea seal and cetacean populations. *Veterinary Record* 138: 647–648.
- TRYLAND, M., L. KLEIVANE, A. ALFREDSSON, M. KJELD, A. ARNASON, S. STUEN, AND J. GODFROID. 1999. Evidence of *Brucella* infection in marine mammals in the North Atlantic Ocean. *Veterinary Record* 144: 588–592.
- VAN BRESSEM, M-F., K. VAN WAEREBEEK, J. A. RAGA, J. GODFROID, S. D. BREW, AND A. P. MACMILLAN. 2001. Serological evidence of *Brucella* species infection in odontocetes from the South Pacific and the Mediterranean. *Veterinary Record* 148: 257–661.

Received for publication 20 May 2005.