

## Should Harbor Seals (*Phoca vitulina*) with Antibodies to *Brucella* Be Rehabilitated?

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### Abstract

#### Introduction

Brucellosis in marine mammals is a poorly understood yet important infectious disease. *Brucella* spp. that differ from the previously recognized species within this genus have been isolated from various marine mammals,<sup>2,4,6,9,13</sup> and there are many more species of marine mammals that are seropositive for *Brucella* antibodies, but from which the organism has not been isolated.<sup>10</sup> Very little is known about the epidemiology, pathogenicity and transmission of these newly recognized *Brucella* spp. despite their potential to cause disease in marine mammals, humans and domestic animals. Infection by marine *Brucella* spp. could be endemic in Pacific harbor seals (*Phoca vitulina richardsi*) in Washington State (USA) and the Province of British Columbia (Canada).<sup>6,7,14</sup> Consequently, stranded harbor seals occasionally test positive for antibodies to *Brucella abortus*, using standard serologic tests developed for cattle. Some of these animals appear healthy; however the presence of anti-*Brucella* antibodies has raised concerns about their suitability for release. Currently, the National Marine Fisheries Service (NMFS) does not have a policy in place regarding testing and interpreting results for *Brucella* spp. in marine mammals. The NMFS Marine Mammal Health and Stranding Response Program does consider *Brucella* a reportable disease and is very interested in developing "best practices" and a policy for testing during rehabilitation and prior to release. Recognizing this and that regions other than the Pacific Northwest also might struggle with this issue,<sup>8</sup> we use peer-reviewed and regional anecdotal information to begin to address the question, "Should harbor seals with antibodies to *Brucella* be rehabilitated?"

All live-stranded harbor seals taken into rehabilitation facilities in Washington State are tested for antibodies to *Brucella abortus* at Washington State Department of Agriculture (Olympia, WA) using the *Brucella* buffered plate agglutination test antigen (BAPA), brucellosis card test using buffered *Brucella* antigen (BBA), the Rivanol precipitation test, and the complement fixation (CF) test. None of these tests have been validated for harbor seals and official guidelines are not available for interpreting brucellosis serology test results from marine mammal sera, however serum samples that are negative by BAPA and BBA are generally considered negative. Samples that are positive for antibodies to *Brucella abortus* on either of these tests are then tested using the CF test and the Rivanol precipitation test. Samples that are positive on either or both of these two serologic tests are considered positive for antibodies to *Brucella* spp. and samples that are negative by CF and Rivanol but positive by BAPA and BBA are considered suspect. Oral, nasal, tracheal, vaginal and anal swabs and feces from live harbor seals with antibodies to

*Brucella* have been submitted to the National Veterinary Services Laboratory (Ames, IA) for *Brucella* culture. Due to the fastidious nature of *Brucella* species during culture attempts however, the lack of culture success for a serologically positive animal may not necessarily prove the animal is not shedding the organism. Some animals have tested negative for antibodies to *Brucella* upon admission to rehabilitation centers, but later tested positive. These could represent cases of community-acquired infection during rehabilitation, immunosuppression at initial testing or a lack of sensitivity in the serologic tests used. Regardless, an animal testing negative once using the standard four panel domestic cattle tests could still be infected with marine *Brucella*. Reliable screening programs for marine mammals do not exist.<sup>8</sup>

#### Wildlife Health Concerns

The primary mission of marine mammal rehabilitation is to release healthy individuals.<sup>5</sup> Since 1994 when marine *Brucella* was described, some harbor seals in Washington State rehabilitation facilities have tested suspect and others positive for antibodies to *Brucella* spp. Serology can reflect exposure, cross-reaction, or active infection. Some animals testing suspect for *Brucella* antibodies have been released without subsequent re-testing or attempts being made to culture *Brucella*. Some animals testing positive have been euthanized without further testing. Other animals testing positive were re-tested and antemortem attempts were made to culture *Brucella* from the animals. In one animal that did not demonstrate rising titers, culture attempts failed to yield *Brucella*, and physical examination and clinical bloodwork failed to find signs of disease. The seal was implanted with a subcutaneous radio-transmitter and released. Several seals that were serologically positive for antibodies to *Brucella* and appeared healthy were humanely euthanized due to concerns about risks associated with release of *Brucella* positive animals. Although physical examination and pre-release clinical pathology results showed these animals to be suitable for release, marine *Brucella* was cultured from various tissues postmortem and necropsies revealed a host of pathological processes that may or may not have been associated with *Brucella* infection.

In work performed in New England, marine *Brucella* was isolated from seals that were not seropositive using standard cattle serologic tests for antibodies to *Brucella abortus*, suggesting a low sensitivity for these tests.<sup>7</sup> Information also is lacking about the specificity of these tests. More information about the pathogenesis of marine *Brucella* in harbor seals and a better understanding of the sensitivity and specificity of tests being currently used (or investigations into the use of a competitive ELISA as previously suggested<sup>8</sup> and used<sup>10</sup>) are needed to better understand when are seropositive seals really infected. Once we develop a means of accurately determining infection, the next question is, "Should positive animals be rehabilitated?" Development of a satisfactory treatment for brucellosis in domestic animals has been difficult. If we agree that all rehabilitated animals should be free of disease prior to release and no satisfactory treatment is developed for brucellosis in harbor seals, than infected seals should be euthanized and not rehabilitated.

On the other hand, if marine *Brucella* infection is endemic in harbor seals in the Pacific Northwest, should infection with marine *Brucella* preclude their release? Harbor seal disease screening work performed on free-ranging harbor seals in the Puget Sound Georgia Basin region by the Washington Department of Fish and Wildlife suggests that infection by marine *Brucella* could be endemic in the population. Serology indicated a prevalence of 47% in yearlings (age 6-18 months) and 37% in subadults (18-48 months).<sup>7</sup> Only 4% of pups (<2 months age), 11% of weaners (age 2-6 months) and 9% of adults (age >48 months) had suspect or positive titers.<sup>7</sup> Based on these data exposure and seroconversion probably occur before six months of age with most animals losing antibodies by 4 years of age.

Assuming such a high percentage of the population is infected with marine *Brucella*, one must wonder if release of a seropositive animal really could start or exacerbate a pre-existing epizootic or be a threat to the seal population at large. Enhanced screening of free-ranging harbor seals is ongoing in the Pacific Northwest and should yield valuable insights into the potential risks posed by release of seropositive animals.

#### Domestic Animal Health Concerns

Considerable effort has been made in the United States to eradicate *Brucella* in domestic animals (*B. abortus*, *B. canis*, *B. melitensis*, *B. ovis*, and *B. suis*) and the discovery of marine *Brucella* spp. in 1994 brought with it concerns about the potential for this organism to cause brucellosis in domestic animals. Experimental infection using marine *Brucella* of different origins, different routes of inoculation and different doses caused abortion and seroconversion in cattle,<sup>12</sup> infection and seroconversion without abortion in sheep,<sup>11</sup> and fulminate infection in guinea pigs.<sup>11</sup> Despite these findings, a harbor seal that has antibodies to *Brucella* and is housed in a rehabilitation center that has proper biosecurity probably represents minimal risk to domestic animals, even if the seal is infected and is shedding bacteria. Similarly, the risk that a single infected seal presents to domestic animals upon its release is probably negligible, when one considers that thousands of infected seals are free-ranging in the region.

#### Zoonotic Risk

Marine *Brucella* spp. are recognized zoonotic pathogens though documented cases are very rare. To date, one case of laboratory-acquired human infection with a marine *Brucella* infection,<sup>1</sup> and two cases of community acquired infection have been reported.<sup>15</sup> Rehabilitation of *Brucella*-positive harbor seals potentially exposes rehabilitators and veterinarians, as well as the general public (post-release) to marine *Brucella*. In a recent case, marine *Brucella* was cultured from the feces of a *Brucella*-positive juvenile harbor seal at Wolf Hollow Wildlife Rehabilitation Center (San Juan Island, Washington), suggesting that some *Brucella*-positive harbor seals are actively shedding *Brucella* organisms. If fecal shedding of the bacterium occurs in other *Brucella*-positive harbor seals, then direct contact and environmental contamination may pose a risk for infection for humans working in rehabilitation centers or the public. Other zoonotic agents occur in pinnipeds and are of concern for rehabilitation staff, including caliciviruses, influenza A and B viruses, parapox virus, B-Hemolytic *Streptococcus* spp., *Clostridium* spp., *Coxiella burnetii*, *Edwardsiella* spp., *Leptospira interrogans*, *Mycoplasma phocacerebrale*, *Mycobacterium* spp., *Vibrio* spp., and *Cryptosporidium parvum*.<sup>3</sup> The risk associated with potential transmission of these pathogens from pinnipeds to humans does not preclude their rehabilitation assuming that proper safety measures are in place at rehabilitation facilities and animals are judged to be free of infectious diseases prior to release.

#### Discussion

Lack of information about the pathogenesis and epidemiology of marine *Brucella* spp. in harbor seals and inadequate or inadequately studied serologic tests make it very difficult to determine if harbor seals that have antibodies to marine *Brucella* should be rehabilitated and released. Serologically positive animals are not always infected or shedding organisms and serologically negative animals can still be infected. Evidence to date suggests that while important and not completely understood, the domestic animal and zoonotic health concerns associated with

rehabilitating harbor seals that have antibodies to *Brucella* are not as great as the individual animal and population-level wildlife health concerns, which require even more information to adequately address.

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