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Evaluating marine *Brucella* infections in cetaceans in the United States

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ABSTRACT

Brucella bacteria species have been documented in the marine environment since the mid-1990s, and exposure to *Brucella* has been documented globally in numerous marine mammal species. Sporadic cases of brucellosis in cetaceans have been reported for animals at display, rehabilitation facilities, and in the wild. Manifestations of infection in cetaceans include late-term abortions, meningoencephalitis, pneumonia, orchitis, and osteomyelitis. The impact of *Brucella* on marine mammal populations is currently unknown. Since 2010 the U.S. National Marine Fisheries Service (NMFS) in cooperation with the National Marine Mammal Stranding Network have been testing numerous samples from cetacean populations along the U.S. coasts and have identified >120 positive animals for marine *Brucella* (by culture and/or PCR), some with clinical signs of brucellosis. These positive animals were found in the Pacific and Atlantic Oceans and the Gulf of Mexico, and consisted of five different small cetacean species (bottlenose dolphin (*Tursiops truncatus*), common dolphin (*Delphinus delphis*), harbor porpoise (*Phocoena phocoena*), Pacific white-sided dolphin (*Lagenodelphis obliquidens*), and striped dolphin (*Stenella coeruleoalba*)). The most commonly affected animals were bottlenose dolphins. Marine-associated brucellosis in humans has not been documented in the U.S. but has been found in four human cases worldwide. Recently marine mammal workers were exposed to a *Brucella* positive porpoise at necropsy but no illness was reported. Increasing reports of positive *Brucella* tissues and brucellosis from marine mammals and especially cetaceans have led to a need to answer key questions about marine *Brucella*. In this paper we will begin to address the presentation of brucellosis (i.e., pathogenic *Brucella* infections) among cetaceans in North America, provide preliminary data on the variety of marine mammal *Brucella* strains circulating in the United States, and address the occupational exposure to *Brucella* from working with marine mammals.

INTRODUCTION

Brucella organisms are gram negative, facultative, intracellular bacteria. To date, ten species of *Brucella* have been identified, two of which are specific to marine mammals (*B. ceti* and *B. pinnipedialis*). Globally, serologic evidence of exposure to *Brucella* spp. has been documented in 14 pinniped and 28 cetacean species (Tryland *et al.* 1999, Nielsen *et al.* 2001, Lynch *et al.* 2011, Nymo *et al.* 2011), and has been isolated from both marine mammal groups

using microbiological techniques. Elk, bison, and feral swine are the primary reservoirs of *B. abortus* and *B. suis* among terrestrial wildlife in North America and these pathogens are largely considered enzootic in certain areas. Among terrestrial species, *Brucella* classically causes late term abortions and placentitis, as well as inflammatory lesions within reproductive organs and decreased milk production.

In cetaceans, *Brucella* has also been associated with abortion, placentitis, endometritis, epididymitis, and orchitis (Ewalt *et al.* 1994, Miller *et al.* 1999, Foster *et al*. 2002, Ohishi *et al*. 2003, Jauniaux *et al.* 2010). Other reported lesions in cetaceans include cutaneous and subcutaneous abscessation, meningoencephalitis, endocarditis, endometritis, hepatitis, pneumonia, splenic necrosis, mastitis and genital ulceration (Gonzales-Barrientos *et al.* 2010, Nymo *et al.* 2011). Skeletal and joint structures are also affected in cases of marine mammal brucellosis and include discospondylitis, osteomyelitis, and bony remodeling (Goertz *et al.* 2011, Nymo *et al.* 2011).

Among pinniped species, no consistent gross pathology attributable to brucellosis has been documented, though the organism has been isolated from feces, urine and tissues (Lambourn *et al*. 2013, Sidor *et al.* 2013) and associated with placentitis in a Northern fur seal (Duncan *et al.* in press). When cattle were experimentally inoculated with *Brucella* isolates obtained from harbor seals (*Phoca vitulina*), seroconversion and abortion were documented (Rhyan *et al.* 2001).

Brucella has also been identified in the uterus and intestinal tracts of lungworms collected from phocids and odontocetes (Garner *et al.* 1997, Perrett *et al.* 2004, Dawson *et al.* 2006, Lambourn *et al.* 2013). Verminous pneumonia is one of the most common pathologies documented in *Brucella*-seropositive harbor seals, though this finding is also relatively common among seronegative seals (Lambourn *et al.* 2013). *Brucella* seropositivity is more common in weaned and juvenile harbor seals in Washington, U.S., in contrast to pups and adults. Hence, ingestion of fish carriers is a proposed route of *Brucella* exposure for this species, though the lifecycles of many marine mammal lungworms are not known, nor is the mechanism by which *Brucella* may be transmitted to fish.

In general, *Brucella* transmission typically occurs through ingestion, inhalation or contact (across mucosal barriers, damaged skin and the conjunctiva) with infected milk, vaginal discharge, lochia, placental tissue, and any aborted materials. *Brucella* is transmitted to offspring transplacentally as well as through lactation (Hernandez-Mora *et al.* 2007, Maquart *et al*. 2009, Gonzalez-Barrientos *et al.* 2010).

Terrestrial mammals are often the source of zoonotic transmission of *Brucella*, though human-to-human transfer has been documented (Godfroid *et al.* 2005). Four human cases of active infections with marine origin (or presumed marine origin) *Brucella* spp. have been described, though none of these individuals had direct exposure to marine mammals (Brew *et al.* 1999, Sohn *et al.* 2003, McDonald *et al.* 2006, Whatmore *et al.* 2008, Macquart *et al*. 2009).

MATERIAL AND METHODS

Cetacean tissue samples were obtained from the National Marine Mammal Stranding Network from cetaceans stranding in the United States. Samples were collected from 2010 to the present. Individual cetaceans were identified for *Brucella* screening if they presented with gross lesions or clinical signs of *Brucella* infection including abnormal joint fluid, skin or lung abscesses, and/or brain lesions including increased cerebrospinal fluid (CSF) and/or abscesses. Joint or tissue samples were sent for polymerase chain reaction (PCR) testing and/or *Brucella* culture. Representative tissue samples were also collected for histopathology and submitted for histopathological evaluation.

Real-time PCR was conducted using primers directed at either the 16s or IS711 gene (Wu *et al.* 2014). *Brucella* culture was performed according to standard methods adapted from Alton et al. 1988. Marine *Brucella* strains were characterized by multi-locus sequence typing (MLST), multiple locus variable number tandem repeat analysis, *Omp* gene, and whole genome sequencing.

Due to the unknown risk that marine *Brucella* poses to marine mammal workers the NMFS Marine Mammal Health and Stranding Response Program (MMHSRP) working with the Centers for Disease Control and Prevention (CDC) and state public health officials assessed the exposure of stranding network personnel to positive *Brucella* animals throughout the United States The CDC worked with the state and local health departments in eight states to contact persons considered potentially exposed to marine *Brucella* species through contact with infected marine mammals

while rescuing, rehabilitating, providing veterinary medical treatment, and performing necropsies. Persons were interviewed to obtain information on dates of exposure, work performed with marine mammals, and type of personal protective equipment worn. Persons classified as being exposed submitted serum samples for testing with the *Brucella* microagglutination test (BMAT) performed at CDC. High-risk activities included use of a bone saw for brain removal and high-pressure hosing of the necropsy room. Persons classified as high-risk were recommended to take antibiotic post-exposure prophylaxis (PEP) consisting of a 3-week course of doxycycline and rifampicin (Sears *et al.* 2012).

RESULTS

Over 120 cetaceans of five species from 17 states have tested positive for marine *Brucella* species from throughout the United States. Preliminary positive cases by region are listed in Figure 1. In the Pacific the most commonly affected species were striped dolphins, whereas bottlenose dolphins predominated along the Atlantic coast and in the Gulf of Mexico.

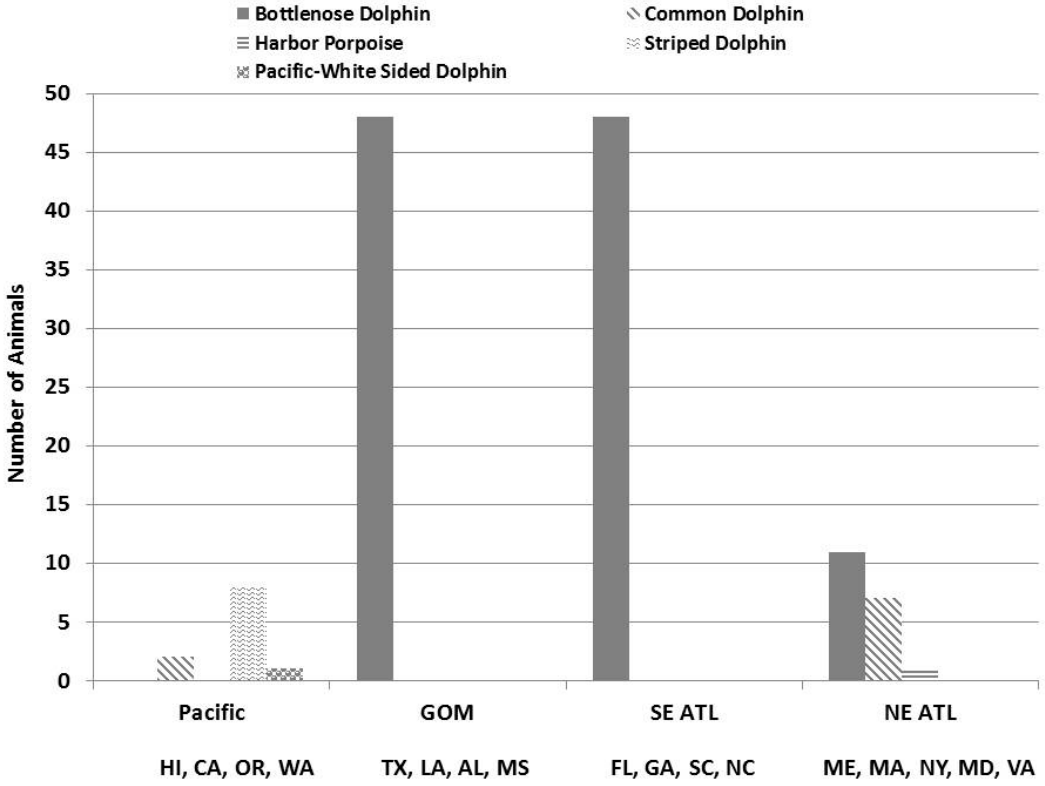

Figure 1: Recent cases of marine *Brucella* infections in stranded cetaceans from 2010-2014 in the United States (culture/PCR positive; n=126).

Gross necropsy findings included infection of the joints, most commonly the scapulo-humeral and occipital joints. Affected joints had large amounts of fluid or exudate ranging from reddish to flocculent to caseous in appearance. In a few cases, lung abscesses, testicular abscesses, or exudate within the uterine horns have been noted. Lastly in some species, and especially in striped dolphins, increase CSF and hydrocephalus were documented as gross necropsy findings. Histopathologic lesions in adult cetaceans primarily consisted of non-suppurative or lymphocytic meningitis or meningoencephalitis. Lymphocyticendometritis and lymphoplasmacytic orchitis have been noted in rare cases.

In adult animals, the most common PCR positive samples were spinal cord, CSF, brain, and joint fluid. Preliminary sequence analysis of the *Omp* gene indicate that multiple genetically distinct types of *Brucella* spp. infect North

American cetaceans with all isolates being most closely related to *B. pinnipedialis* or *ceti*. Similarly, isolates that have been characterized by MLST exhibit sequence types consistent with previously described *Brucella* species derived from marine mammal species. Ongoing whole genome sequencing also indicates there is significant diversity among the *Brucella* species isolated from cetaceans.

The CDC investigation is still ongoing but currently over 60 questionnaires have been administered, 43 persons have submitted serum samples for BMAT testing, and 19 persons have received PEP treatment. Findings to date have found a lack of seropositivity among marine mammal workers who have been tested and no evidence of illness with symptoms compatible with brucellosis reported. Preliminary findings suggest that personal protective equipment (PPE) as laid out in the MMHSRP guidelines and Sears et al. (2012) may be appropriate to protect against exposure to marine *Brucella*. Additionally, marine mammal *Brucella* species may not be readily transmissible to marine mammal workers in non-laboratory settings.

PRELIMINARY CONCLUSIONS

Preliminary findings show that since 2010 multiple stranded small odontocetes in the U.S. have presented with clinical signs of brucellosis, with the predominant species affected being bottlenose, common, and striped dolphins. The most common PCR positive samples were spinal cord, brain, CSF, and joint fluid. Preliminary genetic sequencing of *Brucella* isolates show that they are most closely related to *B. pinnipedialis or ceti* which are consistent with previously described *Brucella* species derived from marine mammal species and there is significant diversity among the *Brucella* species isolated from cetaceans. Research is continuing to better classify the differences in marine *Brucella* species obtained from stranded odontocetes in the U.S.

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