

Brucellosis in Marine Mammals

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Importance

Brucella infections have recently been recognized in seals, sea lions, walruses, dolphins, porpoises, whales and an otter. This organism appears to be widespread in marine mammals, and has probably been endemic in these populations for a long time. The clinical significance is uncertain. A few infections have been associated with placentitis/abortions, neonatal mortality, meningoencephalitis, abscesses or other syndromes, but *Brucella* has also been isolated from normal tissues and asymptomatic animals. There are concerns that brucellosis might affect reproduction or echolocation, particularly in threatened species or naive populations. These concerns are highlighted by the recent isolation of *Brucella* from a newborn Maui's dolphin that died shortly after birth. Maui's dolphins are a highly endangered species, with a population consisting of approximately 100 animals.

Marine mammal isolates of *Brucella* can infect terrestrial mammals, but the frequency of this event is unknown. Some polar bears, which feed on marine mammals, are seropositive for *Brucella*, and there are concerns about possible impacts on this species. Experimental infections in cattle and sheep have been described. Rare human infections have also been documented. One marine mammal isolate caused acute brucellosis in a researcher. Three other infected people had no occupational exposure to marine mammals; two individuals had neurological signs, and the third developed spinal osteomyelitis.

Etiology

Members of the genus *Brucella*, which are facultative intracellular pathogens, are Gram-negative coccobacilli or short rods. *Brucella* strains isolated from marine mammals are genetically distinct from the species isolated from terrestrial mammals.

Formal names have been proposed but not yet accepted for marine *Brucella* isolates. Their naming has been complicated by a debate on *Brucella* nomenclature. The genus *Brucella* has traditionally been classified into six species - *Brucella abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae* - based on each organism's host preference. However, genetic and immunological evidence suggests that all members of the genus *Brucella* are closely related, and some microbiologists have proposed that this genus be reclassified into a single species (*B. melitensis*), with the current species downgraded to biovars. This proposal is controversial, and both taxonomic systems are currently in use. Marine mammal *Brucella* isolates are genetically distinct from terrestrial species, and if the traditional naming system is continued, they are expected to receive species names. *B. maris* was originally suggested for all marine mammal strains of *Brucella*, with division into two or more biovars based on host specificity. (Biovar 1 would contain seal and otter isolates, and biovar 2 would contain cetacean isolates.) A more recent proposal suggests division into at least two species: *B. pinnipediae* for strains from pinnipeds (seals, sea lions and walruses) and *B. cetaceae* for isolates from cetaceans (whales, porpoises and dolphins). Another scheme suggests a division into three distinct groups comprised of isolates from seals, porpoises and dolphins.

Species Affected

Most species of *Brucella* are primarily associated with certain hosts; however, infections can also occur in other species, particularly when they are kept in close contact. Marine mammal isolates of *Brucella* seem to have a host preference for either the order Cetacea or the order Pinnipedia. One isolate was found in an otter, a member of the Mustelidae.

Brucella has been cultured or detected with DNA techniques in many pinniped and cetacean species including common seals/ harbor seals (*Phoca vitulina*), ringed seals (*Phoca hispida*), harp seals (*Phoca groenlandica*), hooded seals (*Cystophora cristata*), grey seals (*Halichoerus grypus*), harbor porpoises (*Phocoena phocoena*), short-beaked common dolphins (*Delphinus delphis*), striped dolphins (*Stenella coeruleoalba*), bottlenose dolphins (*Tursiops truncatus*), Atlantic white-sided dolphins (*Lagenorhynchus acutus*), white beaked dolphins (*Lagenorhynchus albirostris*), Maui's dolphins (*Cephalorhynchus hectori maui*), white-headed dolphins/ Hector's dolphins

(*Cephalorhynchus hectori*), minke whales (*Balaenoptera acutorostrata*) and an European otter (*Lutra lutra*).

Antibodies to *Brucella* have also been detected in many marine mammals. Seropositive species include grey seals (*Halichoerus grypus*), common seals/harbor seals (*Phoca vitulina*), harp seals (*Phoca groenlandica*), ringed seals (*Phoca hispida*), hooded seals (*Cystophora cristata*), Weddell seals (*Leptonychotes weddelli*), Antarctic fur seals (*Arctocephalus gazella*), Hawaiian monk seals (*Monachus schauinslandi*), sea lions (*Neophoca cinerea*), Atlantic walruses (*Odobenus rosmarus rosmarus*), harbor porpoises (*Phocoena phocoena*), Burmeister's porpoises (*Phocoena spinipinnis*), short-beaked common dolphins (*Delphinus delphis*), long-beaked common dolphins (*Delphinus capensis*), striped dolphins (*Stenella coeruleoalba*), bottlenose dolphins (*Tursiops truncatus*), dusky dolphins (*Lagenorhynchus obscurus*), white-headed dolphins/Hector's dolphins (*Cephalorhynchus hectori*), minke whales (*Balaenoptera acutorostrata*), fin whales (*Balaenoptera physalus*), sei whales (*Balaenoptera borealis*), killer whales (*Orcinus orca*), beluga (*Delphinapterus leucas*), narwhal (*Monodon monocerus*), pygmy sperm whales (*Kogia breviceps*) and pilot whales (*Globicephala melas*).

Currently, there is little or no information about the significance of infection in each species. *Brucella* has been found in asymptomatic animals, stranded or dead animals with lesions and, rarely, animals that are ill. *Brucella* may be endemic in populations that have a high seroprevalence, such as hooded seals and dusky dolphins, and an incidental infection in other species.

Terrestrial species can be infected with marine mammal isolates, probably as incidental hosts. Antibodies to *Brucella* have been found in polar bears; these antibodies are thought to result from exposure to infected seals and other prey. Experimental infections with marine mammal isolates have been described in cattle, sheep and guinea pigs, and unpublished experiments suggest that piglets can be infected transiently. Symptomatic infections have also been described in humans.

Geographic Distribution

Brucella appears to be widespread in marine mammal populations. Culture-positive or seropositive animals have been found in the North Atlantic Ocean, the Mediterranean Sea, and the Arctic including the Barents Sea. Infected or exposed animals have also been found along the Atlantic and Pacific coasts of North America; the coasts of Peru, Australia, New Zealand and Hawaii; and in the Solomon Islands and the Antarctic. Most isolates have come from animals in the northern hemisphere, but this may reflect sampling rather than the true distribution of infection. Some authors suspect that *Brucella* is ubiquitous in most marine environments.

Transmission

Transmission of *Brucella* is poorly understood in marine mammals, with little direct evidence to support any route of infection. Terrestrial species of *Brucella* are often transmitted by exposure to the infected placenta, birth fluids and vaginal secretions, and by venereal spread. These routes may also occur in marine mammals; *Brucella* has been isolated from the reproductive organs of cetaceans. Transmission in milk or *in utero* may be possible. The survival of marine isolates in the environment has not been studied; however, terrestrial species of *Brucella* can remain viable for several months in water and on some fomites, particularly when the temperature is low.

Direct or indirect contact among gregarious species could also spread this organism. Fecal shedding of *Brucella* has been described in a harbor seal at a marine mammal rehabilitation center. *Brucella* has been isolated from subcutaneous abscesses, and could theoretically have been inoculated in wounds. However, these abscesses often involve the musculature, with no evidence of trauma; this suggests that they may be caused by hematogenous spread. *Brucella* has also been found in lungworms (*Parafilaroides* sp.) in a harbor seal, and these parasites may act as vectors. Some authors have suggested that marine mammal *Brucella* might be transmitted by the ingestion of infected fish or marine mammals. Support comes from the experimental infection of Nile catfish by a terrestrial species, *B. melitensis*.

The frequency and route of transmission to humans and other terrestrial mammals is unknown. Predation on infected seals has been suggested as a possible route of exposure for polar bears. Cattle have been infected experimentally by intravenous injection, and cattle and sheep by intraconjunctival inoculation. One human infection occurred after exposure in the laboratory, but the source of three other infections is unknown. Humans usually become infected with terrestrial species of *Brucella* by ingesting organisms in food, or by the contamination of mucous membranes and abraded skin.

Incubation Period

The incubation period is unknown.

Clinical Signs

There is little information on the effects of *Brucella* in marine mammals. In terrestrial animals, brucellosis is usually a reproductive disease associated with placentitis, abortion, orchitis and epididymitis. Reproductive disease is difficult to assess in wild marine mammals, but *Brucella* has been isolated from the reproductive organs of some species. In rare cases, infections have also been linked to lesions or clinical disease. *Brucella*-associated abortions and placentitis were reported in two captive bottlenose dolphins. Lesions consistent with a possible abortion were also reported in a wild Atlantic white-sided dolphin. Recently, *Brucella* was isolated from a dead newborn Maui's dolphin in New Zealand; the animal was

born alive but died before taking its first breath. *Brucella*-associated epididymitis has been reported in porpoises, and orchitis from suspected brucellosis was reported in minke whales.

Brucella infections have been linked with systemic disease in a few marine mammals. *Brucella*-associated meningoencephalitis was reported in three stranded striped dolphins. The bacteria were cultured from the brain and appeared to be the primary pathogen. Other signs of *Brucella*-associated systemic disease have been reported mainly in Atlantic white-sided dolphins; the lesions included hepatic and splenic necrosis, lymphadenitis and mastitis. *Brucella* has also been identified as a possible secondary invader or opportunistic pathogen in debilitated seals, dolphins and porpoises. Most *Brucella*-associated lesions reported in porpoises were unlikely to cause death; however, in one animal the infection was associated with hepatic abscesses and peritonitis, as well as epididymitis.

Brucella has been isolated from several subcutaneous abscesses. In addition, this organism has been found in organs with no microscopic or gross lesions, and in apparently healthy animals.

Cattle and sheep have been infected experimentally with marine mammal strains of *Brucella*. Intravenously inoculated cattle aborted. Pregnant sheep and cattle inoculated intraconjunctivally developed only transient infections and did not become ill or abort. Unpublished experiments suggest that piglets are also infected only transiently. Antibodies to *Brucella* have been demonstrated in polar bears, but no disease has been associated with this organism, to date.

Post Mortem Lesions

In dolphins with meningoencephalitis, the lesions were described as severe, chronic, widespread, nonsuppurative meningitis most severe in the brainstem. The meningitis was accompanied by periventricular encephalitis. Moderate to severe fibrosis, inflammatory infiltrates of lymphocytes, plasma cells and macrophages, and vascular damage was reported. In these animals, lesions in organs other than the brain were mild and nonspecific, and consisted of pulmonary congestion and edema, a blubber abscess, gastric erosions, and multiple fractures that may have been the result of terminal trauma. In various marine mammals, *Brucella* has also been associated with subcutaneous abscesses, placentitis/abortion, epididymitis, lymphadenitis, mastitis, spinal discospondylitis, peritonitis, a mineralized lung granuloma, hepatic abscesses, hepatic and splenic necrosis, and macrophage/histiocytic cell infiltration in the liver, spleen and lymph nodes. In some cases, *Brucella* has been recovered from apparently normal tissues.

In minke whales and one Bryde's whale, granular lesions with caseation or calcification have been described in the testes. Microscopic examination

identified the lesions as chronic purulent or granulomatous orchitis. Similar nodular lesions were reported in the uterine endometrium of one female minke whale and the ovary of a pregnant Bryde's whale.

No gross lesions were observed in experimentally infected cattle or their aborted fetuses. Microscopic examination revealed necropurulent placentitis and endometritis in the two animals that aborted, but no lesions in other tissues.

Morbidity and Mortality

Estimates of the prevalence of *Brucella* in marine mammals vary with the species, test conducted, geographic location and population sampled (e.g. stranded animals). One study found that approximately 4% (94 / 2,470) of the marine mammals sampled from the Atlantic, Pacific, and Arctic oceans were seropositive. In smaller studies, seroprevalence rates of 0% to 80% have been reported in various species. Recently, 6% of 119 marine mammals stranded along the Connecticut and Rhode Island coasts were found to be positive in all three serological tests conducted; 48% were positive in one or two of the tests, and were classified as suspects. Most of the suspects and all of the seropositive animals were pinnipeds rather than cetaceans. In particular, 14% (3/21) of the harbor seals and 8% (4/53) of the harp seals were seropositive. In other studies conducted worldwide, particularly high seroprevalence rates have been reported in hooded seals in the North Atlantic Ocean and the Barents Sea (35%); hooded seals from the North Atlantic Ocean (38%); common seals along the coast of Scotland (49%); harbor seals from North America (21%); sea lions in Australia (75%); harbor porpoises along the coast of Scotland (33%); harbor porpoises (31%) and common dolphins (31%) stranded along the English and Welsh coasts; bottlenose dolphins in the Solomon Islands (62-80%, varying with the test); minke whales in the North Pacific (38%); and dusky dolphins (78%), common dolphins (50%), bottlenose dolphins (60%) and Burmeister's porpoises (25%) from the coast of Peru.

The morbidity and mortality rates are unknown. More severe disease might occur in populations where *Brucella* is not endemic. *Brucella*-associated lesions have been reported more frequently in Atlantic white-sided dolphins than most other species, and meningoencephalitis has only been reported in striped dolphins.

Diagnosis

Clinical

No clinical syndrome has been established for brucellosis in marine mammals. Limited evidence suggests that this organism can be considered in abortions, orchitis, epididymitis, abscesses, meningitis/meningoencephalitis and systemic disease. *Brucella* has been found in apparently normal as well as symptomatic animals.

Differential diagnosis

In dolphins with meningitis, the differential diagnosis includes parasitism (*Nasitrema* sp.), staphylococcal infection, and herpesvirus and morbillivirus infections. Other diseases causing abortions, orchitis, epididymitis, abscesses and systemic disease should be considered in marine mammals with these syndromes and evidence of *Brucella* infection.

Laboratory tests

Brucellosis can be diagnosed by culturing the organism from affected animals. Some marine mammal isolates grow poorly on Farrell's medium (FM), a commonly used selective medium for *Brucella*. Although most cetacean isolates become visible on FM after four days of incubation, isolates from seals often grow very slowly and appear in 7 to 10 days, if they grow at all. For this reason, some authors recommend that cultures from marine mammals be incubated for 14 days before being discarded as negative. Concurrent inoculation onto a nonselective medium such as serum dextrose agar or blood agar is also suggested. The recommended incubation conditions for all primary cultures are 10% carbon dioxide at 37°C (98.6°F); most cetacean isolates will grow in the absence of increased CO₂, but most isolates from pinnipeds are capnophilic. Isolates from marine mammals have the typical smooth (S) colony appearance of the genus, and are raised, convex and shiny, with an entire margin. When examined by transmitted light, they are honey colored and translucent.

Brucellae are coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. They are not truly acid-fast but are resistant to decolorization by weak acids; they stain red against a blue background with the Stamp's modification of the Ziehl-Neelsen method. *Brucella* species are usually identified by phage typing and their cultural, biochemical and serological characteristics. These tests, together with a substrate-specific tetrazolium reduction test, can differentiate marine mammal *Brucella* from *Brucella abortus*, *B. suis*, *B. melitensis*, *B. canis* and *B. neotomae*. Care should be taken during identification, as marine mammal isolates are sometimes misidentified initially as terrestrial strains. Cetacean isolates can often be distinguished from pinniped isolates by their CO₂ requirements, their growth on FM in primary culture, and their metabolism of D-galactose. Genetic techniques can also be used to identify marine mammal isolates of *Brucella*.

Serology is generally used in surveillance. It can also be used to screen individual animals, but it is not always reliable. *Brucella* has been cultured from some marine mammals that were seronegative, and seropositive animals are not necessarily infected. The serological tests used in marine mammals have been adapted from livestock *Brucella* tests. They include the buffered *Brucella* antigen tests (rose bengal test and buffered plate agglutination test), serum agglutination tests (tube or

microtiter tests), complement fixation, agar gel immunodiffusion, card agglutination test, rivanol test and enzyme-linked immunosorbent assays (ELISA). In general, these tests have not yet been validated for marine mammals; threshold values have not been established and can vary between laboratories. Serological tests cannot always distinguish reactions to *Brucella* from cross-reactions to other bacteria, particularly *Yersinia enterocolitica* O:9; however, *Y. enterocolitica* O:9 is not known to occur in marine mammals.

Immunostaining has been used to demonstrate *Brucella* in tissues in some research laboratories.

Samples to Collect

Humans have been infected with marine mammal isolates of *Brucella*; samples should be collected and handled with all appropriate precautions.

Brucella has been isolated from all major body tissues in marine mammals. In particular, this organism has been found in the male and female reproductive organs, mammary gland, abscesses, lung and a variety of lymph nodes. Oral, nasal, tracheal, vaginal and anal swabs, as well as feces, can be submitted for culture from live animals. Serum should also be collected for serology. At necropsy, samples should be collected from all tissues with gross lesions. Other samples may also be taken; *Brucella* has been isolated from tissues with microscopic but no gross lesions, as well as tissues without lesions. Blood cultures collected from the heart are occasionally successful at necropsy.

Recommended actions if brucellosis is suspected

Notification of authorities

Brucella has been isolated from marine mammals in the U.S. State authorities should be consulted for reporting requirements in each state. The National Marine Fisheries Service (NMFS) Marine Mammal Health and Stranding Response Program considers *Brucella* a reportable disease.

Federal: Area Veterinarians in Charge (AVIC)

www.aphis.usda.gov/animal_health/area_offices.htm

State Veterinarians:

www.aphis.usda.gov/vs/sregs/official.html

Control

Specific control methods have not been established for brucellosis in marine mammals. General principles of infection control including isolation, disinfection and good hygiene should be used with infected animals in marine mammal facilities. Some authors suggest that centers involved in marine mammal rehabilitation should routinely screen animals for *Brucella*.

In general, *Brucella* species are readily killed by most commonly available disinfectants including hypochlorite solutions, 70% ethanol, isopropanol, iodophores, phenolic

disinfectants, formaldehyde, glutaraldehyde and xylene; however, organic matter and low temperatures decrease the efficacy of disinfectants. Disinfectants reported to destroy *Brucella* on contaminated surfaces include 2.5% sodium hypochlorite, 2-3% caustic soda, 20% freshly slaked lime suspension, or 2% formaldehyde solution (all tested for one hour). Ethanol, isopropanol, iodophores, substituted phenols or diluted hypochlorite solutions can be used on contaminated skin. Alkyl quaternary ammonium compounds are not recommended for this purpose. Autoclaving [moist heat of 121°C (250°F) for at least 15 minutes] can be used to destroy *Brucella* species on contaminated equipment. These organisms can also be inactivated by dry heat [160-170°C (320-328°F) for at least 1 hour]. Boiling for 10 minutes is usually effective for liquids. Xylene (1ml/liter) and calcium cyanamide (20 kg/m³) are reported to decontaminate liquid manure after 2 to 4 weeks. *Brucella* species can also be inactivated by gamma irradiation (e.g. in colostrum) and pasteurization. *Brucella* survives for very short periods in meat, unless it is frozen; in frozen meat, survival times of years have been reported.

Public Health

Marine mammal *Brucella* can infect humans. People who hunt marine mammals may be at increased risk of exposure, particularly when dressing carcasses or consuming raw meat. Other groups at risk may include veterinarians, zoologists, laboratory workers, fishermen, and people who work in marine mammal rehabilitation or display centers, as well as anyone who approaches a beached animal or carcass on a beach. As of July 2007, very few human infections have been reported; these infections may either be rare or underdiagnosed. One infection occurred in a researcher exposed in the laboratory. The symptoms included headaches, fatigue and severe sinusitis, and resolved completely after antibiotic treatment. Two patients with community-acquired neurobrucellosis and intracerebral granulomas were reported in the U.S. One person had a three-month history of periorbital pain, headaches and periodic seizures. The other had a one-year history of headaches, nausea, vomiting and progressive deterioration in eyesight. The source of infection could not be determined in either case, but both patients had recently emigrated from Peru and regularly consumed raw fish (in cerviche) and unpasteurized cheese. One had no significant exposure to marine mammals; the other regularly swam in the ocean but had not been directly exposed to marine mammals. The fourth case occurred in New Zealand, in a man with a two-week history of spinal osteomyelitis characterized by fever, rigors and tenderness in the lumbar region of the spine. This patient had not been exposed to marine mammals, but he was a fisherman who had regular contact with uncooked fish bait and raw fish. He had also eaten raw freshly caught fish.

Zoonotic infections with marine mammal strains may be similar to infections with terrestrial strains. In

humans, most species of *Brucella* cause similar syndromes. Infections can be either asymptomatic or symptomatic. In symptomatic cases, the disease is extremely variable and the clinical signs may appear insidiously or abruptly. Typically, human brucellosis begins as an acute febrile illness with nonspecific flu-like signs such as fever, headache, malaise, back pain, myalgia and generalized aches. Drenching sweats can occur, particularly at night. Some patients recover spontaneously, while others develop persistent symptoms that typically wax and wane. Occasionally seen complications include arthritis, spondylitis, chronic fatigue, and epididymo-orchitis. Neurologic signs (including personality changes, meningitis, uveitis and optic neuritis), anemia, internal abscesses, nephritis, endocarditis and dermatitis can also occur. Neurological signs usually occur in less than 5% of patients. Other organs and tissues can also be affected, resulting in a wide variety of syndromes. Treatment is with antibiotics; however, relapses can be seen months after the initial symptoms, even in successfully treated cases. The mortality rate is low; in untreated persons, estimates of the case fatality rate vary from less than 2% to 5%. Deaths are usually caused by endocarditis or meningitis.

Internet Resources

Centers for Disease Control and Prevention (CDC).

Brucellosis.

http://www.cdc.gov/ncidod/dbmd/diseaseinfo/brucellosis_t.htm

Public Health Agency of Canada. Material Safety

Data Sheets

<http://www.phac-aspc.gc.ca/msds-ftss/index.html>

The Merck Manual

<http://www.merck.com/pubs/mmanual/>

The Merck Veterinary Manual

<http://www.merckvetmanual.com/mvm/index.jsp>

The National Marine Fisheries Service (NMFS) Marine

Mammal Health and Stranding Response Program

<http://www.nmfs.noaa.gov/pr/health/>

World Organization for Animal Health (OIE)

<http://www.oie.int>

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*Link defunct as of 2007