Slide 1

Brucellosis in Marine Mammals

Slide 2

Overview

- Organism
- History
- Epidemiology
- Transmission
- Disease in Humans
- Disease in Animals
- Prevention and Control
- Actions to take

In today's presentation we will cover information regarding the organism that causes marine brucellosis and its epidemiology. We will also talk about the history of the disease, how it is transmitted, species that it affects (including humans), and clinical and necropsy signs observed. Finally, we will address prevention and control measures for marine brucellosis, as well as actions to take if marine brucellosis is suspected.

Photos: (Top) Bowhead whales; (Bottom) killer whales. Source: NOAA]

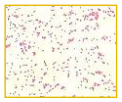
Slide 3

THE ORGANISM

Slide 4

The Organism

- Gram negative, coccobacillus
  - Facultative, intracellular organism
- Marine mammal strains distinct from terrestrial mammal strains
- Environmental persistence
  - Little is known
  - Thought to be persistent



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. *Brucella* species can persist in the environment invariably depending on temperature, pH, and humidity.

[Photo: Micrograph of *Brucella* organisms. *Brucella* spp. are gram-negative in their staining morphology. *Brucella* spp. are poorly staining, small gram-negative coccobacilli (0.5-0.7 x 0.6-1.5 µm), and are seen mostly as single cells and appearing like "fine sand". Source: CDC Public Health Image Library #1901]

Slide 5

Brucella Nomenclature

- Six species (terrestrial mammals)
- Marine mammals
  - Proposed names only
  - *B. pinnipediae*
    - Seals, sea lions, walruses
  - *B. cetaceae*
    - Whales, porpoises, dolphins

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. A recent proposal for marine mammal strains 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.

[Photos: (Top) Ringed seal; (Bottom) bottlenose dolphin. Source: NOAA]

Slide 6

HISTORY

Slide 7

History of Brucellosis

- Early 1800s
  - Sir William Burnett differentiates fevers affecting seamen in the Mediterranean
- Late 1880s
  - Sir David Bruce isolates the cause of Malta fever, *Micrococcus melitensis*
  - Dr. Bernhard Band discovers cause of cattle abortion in Denmark, *Bacterium abortus* ("Bang's disease")

Sir William Burnett was a physician to the British Navy in 1810 and was the first person to differentiate between the various fevers affecting seamen in the Mediterranean. It is thought that Malta became such an important center for the study of undulant fever because many British troops were sent there to recuperate following the Crimean War (1853-1856), along with skillful medical doctors utilizing clinical thermometers to monitor the disease progression. The microorganism responsible for Malta fever was discovered by a British Army physician, Sir David Bruce, on July 9, 1887, which he called *Micrococcus melitensis*. It was isolated from the spleen of a British soldier who had died of the disease. He later established goats as the main reservoir for infection by identifying the organism in their blood, urine, and milk. A Danish physician and veterinarian, Bernhard Bang discovered *Bacterium abortus* in 1897 while investigating contagious abortion that had been affecting cattle in Denmark for over a century. He also discovered the organism affected horses, sheep, and goats. Thus the disease became known as "Bang's disease". The connection between animals and humans was discovered by Alice Evans, an American bacteriologist in the 1920s.

Slide 8

History of Brucellosis

- *Brucella* spp. first isolated from marine mammals in 1994
  - Scotland: harbour seal, harbour porpoise, common dolphin
  - California: aborted fetus from a captive bottlenose dolphin
- Serological evidence
  - Indicates worldwide exposures

Brucella species were first isolated from marine mammals in 1994. Mammals affected included a harbour seal (*Phoca vitulina*), a harbour porpoise (*Phocoena phocoena*) and a common dolphin *(Delphinus delphis*) in Scotland, and an aborted fetus from a captive bottlenose dolphin (*Tursiops truncatus*) in California. Since then, *Brucella* species have been isolated from many other marine mammals. In addition, serological evidence has demonstrated previous infection in a range of marine mammals worldwide.

Source: IH Nymo, M Tryland, and J Godfroid. A review of *Brucella* infection in marine mammals, with special emphasis on *Brucella pinnipedialis* in the hooded seal (*Cystophora cristata*). Veterinary Research 2011, 42:93.

Slide 9

EPIDEMIOLOGY

Slide 10

Geographic Distribution

- Worldwide distribution
  - North Atlantic Ocean
  - Mediterranean Sea
  - Arctic, including Barents Sea
  - Atlantic and Pacific coasts of North America
  - Coasts of Peru, Australia, New Zealand, Hawaii, Solomon Islands, Antarctic

Marine brucellosis seems to have a worldwide geographic distribution. 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. Some authors suspect that *Brucella* is ubiquitous in most marine environments.

[Photo: Atlantic spotted dolphins. Source: NOAA]

Slide 11

Species Affected

- Culture confirmed/DNA detected
  - Seals, porpoises, dolphins, minke whales, otters
- Antibodies detected
  - Seals, sea lions, walruses, porpoises, dolphins, minke whales, fin whales, killer whales, beluga whales, narwhal, pygmy sperm whales, pilot whales
- Also found in asymptomatic animals

Brucella has been cultured or detected with DNA techniques in many pinniped and cetacean species. Antibodies to *Brucella* have also been detected in many marine mammals. 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.

Slide 12

Brucella in Humans

- Occupational risk
  - Laboratory workers
  - Veterinarians
  - Zoologists
  - Fishermen
  - Rehabilitators
- Hunters
- Travelers

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.

[Photo: Marine mammal worker. Sources Deborah Gabris/UC Davis CVM Occupational Safety]

Slide 13

TRANSMISSION

Slide 14

Transmission in Marine Mammals

- Contact with infected placenta, birth fluids, vaginal secretions
- Venereal
- Milk
- *In utero*
- Fecal contact
- Vectors
- Ingestion of infected fish, mammals

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.

[Photo: California sea lion eating salmon. Source: Oregon Dept. of Fish and Wildlife]

Slide 15

Transmission in Humans and Other Animals

- Frequency and route of transmission unknown
  - Polar bear exposure
  - Experimental infection of cattle
  - Human laboratory exposure
- Humans usually infected with terrestrial species via:
  - Ingestion, contamination of mucous membranes or abraded skin

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.

Slide 16

DISEASE IN HUMANS

Slide 17

Clinical Signs in Humans

- Similar to terrestrial *Brucella* strains
  - Acute febrile illness
  - Non-specific influenza-like signs
  - May wax and wane
- Complications
  - Arthritis, spondylitis, chronic fatigue, epididymo-orchitis, neurological, anemia, abscesses, other

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. 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.

Slide 18

Marine Brucellosis: Human Case Examples

- Laboratory exposure
- Natural infections
  - Neurobrucellosis
    - History of raw fish and unpasteurized cheese consumption
  - Spinal osteomyelitis
    - Fisherman
    - History of raw fish contact and consumption

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. 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 ceviche) 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.

[Photo: Fisherman. Source: NOAA]

Slide 19

Treatment and Prognosis in Humans

- Treatment
  - Antibiotics
  - Relapses possible
- Prognosis for marine mammal brucellosis unclear
  - Likely similar to terrestrial strains
  - Low mortality rate: 2 to 5%
  - Death due to endocarditis, meningitis

Treatment is with antibiotics; however, relapses can be seen months after the initial symptoms, even in successfully treated cases. Although not much is known about marine brucellosis in humans, it may be like other brucellosis strains. In other *Brucella* species, 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.

Slide 20

DISEASE IN ANIMALS

Slide 21

Clinical Signs in Animals

- Reproductive disease
  - Abortion, placentitis, epididymitis, etc.
- Systemic disease
  - Meningoencephalitis
  - Hepatic and splenic necrosis
  - Lymphadenitis
- Secondary invader
- May be asymptomatic

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 (abortion, placentitis, epididymitis, orchitis). *Brucella* infections have been linked with systemic disease in a few marine mammals. *Brucella*-associated meningoencephalitis was reported in three stranded striped dolphins. 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. *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.

Slide 22

Postmortem Lesions

- Reproductive organs
  - Placentitis/abortion
  - Epididymitis/orchitis
  - Mastitis
- Other
  - Subcutaneous abscesses
  - Peritonitis
  - Lung granulomas
  - Hepatic and splenic necrosis

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.

Slide 23

Morbidity and Mortality

- Unknown morbidity and mortality
  - Likely most severe in non-endemic areas

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.

[Photo: Striped dolphin. Source: NOAA]

Slide 24

Differential Diagnosis

- Parasitism
- Staphylococcal infection
- Herpesvirus
- Morbillivirus
- Other diseases causing abortion, orchitis, epididymitis, abscesses, and systemic disease

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.

Slide 25

Samples to Collect

- Reproductive organs
- Lymph nodes
- Lungs
- Swabs
  - Oral, nasal, tracheal vaginal, anal, fecal
- Serum
- All gross lesions

Marine brucellosis is pathogenic to humans and care should be taken when collecting samples for laboratory diagnosis. 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.
[Photo: Diagnostic swab. Source: Danelle Bickett-Weddle/CFSPH]

Slide 26

Laboratory Diagnosis

- Culture
  - Phage typing
  - Biochemical characteristics
  - Genetic techniques (e.g., sequencing)
- Serology
  - Useful for surveillance
  - Various tests adapted from livestock *Brucella* tests
  - Not validated for marine mammals

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. *Brucella* species are usually identified by phage typing and their cultural, biochemical and serological characteristics. Genetic techniques can also be used to identify marine mammal isolates of *Brucella*.

Serology is generally used in surveillance. 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.

Slide 27

PREVENTION AND CONTROL

Slide 28

Recommended Actions

- Notification of authorities
  - Federal Area Veterinarian in Charge (AVIC)
    http://www.aphis.usda.gov/animal_health/area_offices/
  - State veterinarian
    http://www.aphis.usda.gov/emergency_response/downloads/nahems/fad.pdf

The National Marine Fisheries Service (NMFS) Marine Mammal Health and Stranding Response Program considers *Brucella* a reportable disease. State and/or federal authorities should be consulted for specific guidelines.

Slide 29

Prevention and Control

- Specific control methods not established for marine mammals
- General principles of infection control
  - Isolation
  - Disinfection
  - Good hygiene
- Routine screening of animals in rehabilitation?

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.*

Slide 30

Prevention and Control

- Readily killed by most disinfectants
  - Hypochlorite
  - 70% ethanol
  - Isopropanol
  - Iodophores
  - Phenolics
  - Formaldehyde/glutaraldehyde
- Quaternary ammonium compounds not recommended

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. Alkyl quaternary ammonium compounds are not recommended. Autoclaving [moist heat of 121°C (250°F) for at least 15 minutes] can be used to destroy *Brucella* species on contaminated equipment.

[Photo: Disinfectant bottles. Source: Dani Ausen/CFSPH]

Slide 31

Prevention and Control

- Education about risk of transmission
  - Veterinarians, hunters, rehabilitators, marine animal facility workers
- Wear proper attire if dealing with infected animals/tissues
  - Gloves, masks, goggles

Educate those at greatest risk about the routes of transmission of brucellosis. Those who have the greatest occupational exposure are people who can come into contact with infected animals or tissues from infected animals. These include veterinarians, hunters, marine rehabilitators, and people who work at marine animal facilities. Properly protecting yourself, if you are an "at risk" individual, by wearing gloves, masks, goggles, and coveralls to prevent exposure to tissues and body secretions of infected animals can help.

Slide 32

Additional Resources

- Center for Food Security and Public Health
  - www.cfsph.iastate.edu
- CDC Brucellosis
  - http://www.cdc.gov/ncidod/dbmd/diseaseinfo/brucellosis_g.htm

Slide 33

Acknowledgments

Development of this presentation was made possible through grants provided to the Center for Food Security and Public Health at Iowa State University, College of Veterinary Medicine from the Centers for Disease Control and Prevention, the U.S. Department of Agriculture, the Iowa Homeland Security and Emergency Management Division, and the Multi-State Partnership for Security in Agriculture.

Authors: *Kerry Leedom Larson, DVM, MPH, PhD, DACVPM; Anna Rovid Spickler, DVM, PhD; Sarah Viera, MPH*
Reviewer: *Glenda Dvorak, DVM, MPH, DACVPM*

Last updated: January 2012