Brucellosis

Undulant Fever,
Malta Fever,
Mediterranean Fever,
Enzootic Abortion,
Epizootic Abortion,
Contagious Abortion,
Bang's Disease

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Importance

Brucellosis, a bacterial disease caused by members of the genus *Brucella*, is an important zoonosis and a significant cause of reproductive losses in animals. Brucellosis is usually caused by *Brucella abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs. Abortions, placentitis, epididymitis and orchitis are the most common consequences, although other syndromes are also reported. The main impact is economic; deaths are rare except in the fetus and neonate. Some *Brucella* species are also maintained in wildlife populations. Wildlife reservoirs including feral pigs, bison, elk and European hares complicate eradication efforts for *B. abortus* and *B. suis*. Marine mammal isolates of *Brucella* have recently been recognized in many species of pinnipeds and cetaceans, and there are concerns that these organisms might have a detrimental impact on some species.

Most species of *Brucella* can infect animals other than their preferred hosts, when they come in close contact. *B. abortus*, *B. melitensis*, *B. suis*, *B. canis* and marine mammal *Brucella* species are human pathogens. In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs. Most cases are caused by occupational exposure to infected animals or the ingestion of unpasteurized dairy products. In the U.S., *B. suis* has been eliminated from commercial pigs and *B. abortus* has nearly been eradicated from domesticated ruminants. As a result, human brucellosis is rare. However, this disease remains a common and serious problem in some parts of the world. In addition, some species of *Brucella* could be used in a bioterrorist attack.

Etiology

Brucellosis results from infection by various species of *Brucella*, a Gram negative, facultative intracellular coccobacillus or short rod in the family Brucellaceae. Six named species occur in animals: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis* and *B. neotomae*. One or more unnamed species of *Brucella* have been found in marine mammals. Formal names proposed for marine mammal isolates are *B. maris* for all strains, or *B. pinnipediae* for strains from pinnipeds (seals, sea lions and walruses) and *B. cetaceae* for isolates from cetaceans (whales, porpoises and dolphins). Some species of *Brucella* contain biovars. Five biovars have been reported for *B. suis*, three for *B. melitensis*, and up to nine for *B. abortus*.

Each *Brucella* species is associated most often with certain hosts. *B. abortus* usually causes brucellosis in cattle, bison and buffalo. *B. melitensis* is the most important species in sheep and goats, but *B. ovis* can also cause infertility in rams. *B. canis* causes disease almost exclusively in dogs. *B. neotomae* is found in rodents, but has not been linked to disease. *B. suis* contains more diverse isolates than other *Brucella* species, and these isolates have broader host specificity. *B. suis* biovars 1, 2 and 3 are maintained in pigs; European hares are also a reservoir for biovar 2. Biovar 4 mainly affects reindeer and caribou, and is not normally found in pigs. This biovar was formerly known as *B. rangiferi*. Biovar 5 occurs in rodents.

In humans, brucellosis can be caused by *B. abortus*, *B. melitensis*, *B. suis* biovars 1-4 and, rarely, *B. canis* or marine mammal *Brucella*. Live vaccines for *B. abortus* and *B. melitensis*, as well as the *B. canis* M- strain (a less virulent strain used as an antigen for serological testing), are also pathogenic for humans. *B. ovis*, *B. neotomae* and *B. suis* biovar 5 have not been linked to human disease.

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*), which contains many biovars. This proposal is controversial, and both taxonomic systems are currently in use. The multiple species nomenclature is used in this factsheet.

Geographic Distribution

Brucellosis is found worldwide but it is well controlled in most developed countries. Clinical disease is still common in the Middle East, Asia, Africa, South and Central America, the Mediterranean Basin and the Caribbean.

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Brucella species vary in their geographic distribution. *B. abortus* is found worldwide in cattle-raising regions except in Japan, Canada, some European countries, Australia, New Zealand and Israel, where it has been eradicated. Eradication from domesticated herds is nearly complete in the U.S. *B. abortus* persists in wildlife hosts in some regions, including the Greater Yellowstone Area of North America. *B. melitensis* is particularly common in the Mediterranean. It also occurs in the Middle East and Central Asia, around the Persian Gulf (also known as the Arabian Gulf) and in some countries of Central America. This organism has been reported from Africa and India, but it does not seem to be endemic in northern Europe, North America (except Mexico), Southeast Asia, Australia or New Zealand. *B. ovis* probably occurs in most sheep-raising regions of the world. It has been reported from Australia, New Zealand, North and South America, South Africa and many countries in Europe.

In the past, *B. suis* was found worldwide in swine-raising regions. This organism has been eradicated from domesticated pigs in the U.S., Canada, many European countries and other nations. However, it persists in wild and/ or feral swine populations in some areas, including the U.S., Europe and Queensland, Australia. Sporadic outbreaks are reported in domesticated herds or humans due to transmission from this source. *B. suis* continues to occur in domesticated herds in some countries of South and Central America (including Mexico) and Asia. *B. suis* biovars 1 and 3 are found worldwide, but other biovars have a limited geographic distribution. Biovar 2 occurs in wild boar in much of Europe. Biovar 4 (rangiferine brucellosis) is limited to the Arctic regions of North America and Russia including Siberia, Canada and Alaska. Biovar 5 (murine brucellosis) is found in the former USSR.

B. canis probably occurs throughout most of the world; however, New Zealand and Australia appear to be free of this organism. *Brucella* species also seem 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.

Transmission

B. abortus, *B. melitensis*, *B. suis* and *B. canis* are usually transmitted between animals by contact with the placenta, fetus, fetal fluids and vaginal discharges from an infected animal. Animals are infectious after either an abortion or full term parturition. Although ruminants are usually asymptomatic after their first abortion, they can become chronic carriers, and continue to shed *Brucella* in milk and uterine discharges during subsequent pregnancies. Dogs may also shed *B. canis* in later pregnancies, with or without symptoms. Entry into the body occurs by ingestion and through the mucous membranes, broken skin and possibly intact skin.

Most or all *Brucella* species are also found in semen. Males can shed these organisms for long periods or lifelong. The importance of venereal transmission varies with the species. It is the primary route of transmission for *B. ovis*. *B. suis* and *B. canis* are also spread frequently by this route. *B. abortus* and *B. melitensis* can be found in semen, but venereal transmission of these organisms is uncommon. Some *Brucella* species have also been detected in other secretions and excretions including urine, feces, hygroma fluids, saliva, and nasal and ocular secretions. In most cases, these sources seem to be relatively unimportant in transmission; however, some could help account for direct non-venereal transmission of *B. ovis* between rams.

Brucella can be spread on fomites including feed and water. In conditions of high humidity, low temperatures, and no sunlight, these organisms can remain viable for several months in water, aborted fetuses, manure, wool, hay, equipment and clothes. *Brucella* can withstand drying, particularly when organic material is present, and can survive in dust and soil. Survival is longer when the temperature is low, particularly when it is below freezing.

Accidental hosts usually become infected after contact with maintenance hosts. Although the ruminant udder is usually colonized during the course of an infection, it can also be infected by direct contact (for example, by bacteria on the hands of farm workers). This can result in the long-term shedding of species not normally found in ruminant milk, such as *B. suis*. Humans usually become infected by ingesting organisms or by the contamination of mucous membranes and abraded skin. In the laboratory and probably in abattoirs, *Brucella* can be transmitted in aerosols. Common sources of infection for people include contact with animal abortion products; ingestion of unpasteurized dairy products from cows, small ruminants or camels; ingestion of undercooked meat, bone marrow or other uncooked meat products; contact with laboratory cultures and tissue samples; and accidental injection of live brucellosis vaccines. Human to human transmission is rare, but has been reported after blood transfusion, bone marrow transplantation or sexual intercourse. Rare congenital infections seem to result from transplacental transmission or the ingestion of breast milk. Congenital infections might also occur if the infant is exposed to organisms in the mother's blood, urine or feces during delivery.

Disinfection

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

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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 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 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. Their persistence in unpasteurized cheese is influenced by the type of fermentation and ripening time. The fermentation time necessary to ensure safety in ripened, fermented cheeses in unknown, but is estimated to be approximately three months. *Brucella* is reported to persist for weeks in ice cream and months in butter. This organism survives for very short periods in meat, unless it is frozen; in frozen meat, survival times of years have been reported.

Infections in Humans

Incubation Period

The incubation period is difficult to determine in humans but has been estimated at five days to three months. Most infections seem to become apparent within two weeks. Aerosolization of bacteria in biological weapons could result in a shorter incubation period.

Clinical Signs

Brucellosis is a multisystemic disease with a broad spectrum of symptoms. Asymptomatic infections are common. In symptomatic cases, the disease is extremely variable and the clinical signs may appear insidiously or abruptly. Typically, 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. Splenomegaly, hepatomegaly, coughing and pleuritic chest pain are sometimes seen. Gastrointestinal signs including anorexia, nausea, vomiting, diarrhea and constipation occur frequently in adults but less often in children.

In many patients, the symptoms last for two to four weeks and are followed by spontaneous recovery. Others develop an intermittent fever and other persistent symptoms that typically wax and wane at 2 to 14 day intervals. Most people with this undulant form recover completely in three to 12 months. A few patients become chronically ill. Relapses can occur months after the initial symptoms, even in successfully treated cases. Hypersensitivity reactions can mimic the symptoms of brucellosis.

Complications are seen occasionally, particularly in the undulant and chronic forms. The most common complications are arthritis, spondylitis, epididymo-orchitis and chronic fatigue. Neurological signs occur in up to 5% of cases. They may include personality changes, meningitis, encephalitis and peripheral neuropathy. Uveitis, optic neuritis and papilledema have been reported. Endocarditis is one of the most serious complications, and is often the cause of death in fatal cases. Many other organs and tissues can also be affected, resulting in a wide variety of syndromes including nephritis, dermatitis, vasculitis, lymphadenopathy, deep vein thrombosis, granulomatous hepatitis, cholecystitis, osteomyelitis, anemia, leukopenia and thrombocytopenia. Abscesses can occur in internal organs.

The symptoms of congenital brucellosis are variable. Some congenitally infected infants are delivered prematurely, while others are born at full term. Common symptoms include low birth weight, fever, failure to thrive, jaundice, hepatomegaly and splenomegaly. Some newborns with congenital brucellosis have respiratory difficulty or severe respiratory distress, hypotension, vomiting and other signs of sepsis. Other infants may be asymptomatic or have only mild symptoms at birth. Whether brucellosis can lead to spontaneous abortion in humans is controversial.

Communicability

Brucellosis is not usually transmitted from person to person. Rarely, bacteria have been transmitted by bone marrow transplantation, blood transfusion or sexual intercourse. Rare congenital infections have also been documented. In some cases, the infant appeared to be infected through the placenta, and in others by the ingestion of breast milk. Brucellosis was reported in an obstetrician who swallowed secretions while trying to clear a congenitally infected infant's respiratory tract at birth.

Diagnostic Tests

Microscopic examination of stained smears can be useful for a presumptive diagnosis, particularly if the direct examination is supported by other tests. Brucellae are coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. They are not truly acid-fast; however, they are resistant to decolorization by weak acids, and stain red against a blue background with the Stamp's modification of the Ziehl-Neelsen method. Other organisms such as *Coxiella burnetii* can resemble *Brucella*.

In humans, the definitive diagnosis is by culture or serology. *Brucella* species can sometimes be isolated from the blood early in the infection; bone marrow is often positive at this stage. Occasionally, bacteria can be recovered from the cerebrospinal fluid, urine or tissues. *Brucella* spp. can be isolated on a variety of plain media, or selective media such as Farrell's medium or Thayer-Martin's modified medium. Enrichment techniques can also be used. Colony morphology varies with the species. Colonies of smooth forms (*B. abortus*, *B. suis*, *B. melitensis* and marine mammal *Brucella*) are round with smooth

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margins. When the plates are viewed in daylight through a transparent medium, these colonies are translucent and a pale honey color. From above, they are convex and pearly white. *B. ovis* and *B. canis* are rough (R) forms. The colonies are round, shiny and convex, but their rough nature can be seen by examining the colony with oblique illumination. Most *Brucella* species form colonies within a few days, but isolates from seals grow slowly and may take 7 to 10 days to become visible on selective media. *Brucella* isolates can be identified to the species and biovar level by phage typing and cultural, biochemical and serological characteristics. Care should be taken during identification, as marine mammal isolates are sometimes misidentified initially as terrestrial strains. Genetic techniques can also be used for biotyping.

Most human infections are diagnosed by serology. Tests used include serum agglutination, a modified Coombs' (antiglobulin) technique, ELISAs and immunoblotting (Western blotting). Serologic diagnosis is complicated by previous exposures and other factors; a definitive diagnosis usually requires a fourfold rise in titer. Immunostaining can sometimes demonstrate the presence of *Brucella* spp. in a clinical specimen. PCR techniques can also be used for diagnosis. Chronic brucellosis can be extremely difficult to diagnose, if the serologic results are equivocal and the organism cannot be cultured.

Treatment

Antibiotics are usually the mainstay of treatment; long-term treatment may be required. Some forms of localized disease, such as endocarditis, may require surgery.

Prevention

Human brucellosis is usually prevented by controlling the infection in animals. Pasteurization of dairy products is an important safety measure where this disease is endemic. Unpasteurized dairy products and raw or undercooked animal products (including bone marrow) should not be consumed.

Good hygiene and protective clothing/equipment are very important in preventing occupational exposure. Precautions should be taken to avoid contamination of the skin, as well as inhalation or accidental ingestion of organisms when assisting at a birth, performing a necropsy, or butchering an animal for consumption. Particular care should be taken when handling an aborted fetus or its membranes and fluids. Risky agricultural practices such as crushing the umbilical cord of newborn livestock with the teeth or skinning aborted fetuses should be avoided. The Strain 19 *B. abortus* vaccine and *B. melitensis* Rev-1 vaccine must be handled with caution to avoid accidental injection or exposure. Adverse events have also been reported with the *B. abortus* RB51 vaccine, although it is safer than Strain 19. Persistent infections with vaccine strains have occasionally been reported in vaccinated animals. These strains can be shed in the milk or aborted fetuses and can infect humans. Obstetricians should also take precautions when assisting at human births, particularly in regions where brucellosis is common. Recently, an obstetrician became infected by ingesting amniotic fluid and secretions from a congenitally infected infant. In the laboratory, *Brucella* spp. should be handled under biosafety level 3 conditions or higher. Human vaccines are not available.

Morbidity and Mortality

Brucellosis is usually an occupational disease; most cases occur in abattoir workers, veterinarians, hunters, farmers, reindeer/caribou herders and livestock producers. Brucellosis is also one of the most easily acquired laboratory infections. People who do not work with animals, tissues or bacterial cultures usually become infected by ingesting unpasteurized dairy products. Other cultural practices, such as eating bone marrow from reindeer and caribou incidentally infected with *B. suis*, are risk factors in some populations. In endemic areas, the reported incidence ranges from fewer than 0.01 to more than 200 cases per 100,000 population. Human brucellosis is rare in the U.S.; the annual incidence is less than 0.5 cases per 100,000 persons; approximately 100 cases have been reported annually for the past ten years. However, some studies suggest that this disease is underdiagnosed and underreported in the U.S.

Many human infections are asymptomatic or self-limiting; however, some symptomatic infections can be prolonged, with slow recovery and a small possibility of complications. Increased numbers of symptomatic infections could be seen after a biological attack with aerosolized bacteria. The incidence and severity of disease varies with the species of *Brucella*. *B. melitensis* is considered to be the most severe human pathogen in the genus. *B. abortus* and *B. suis* biovars 1, 3 and 4 are also important human pathogens. *B. suis* biovar 2 and *B. canis* infections are rarely reported in humans. However, serologic studies have reported antibodies to *B. canis* in 13% of hospital patients in Mexico, 0.3% of sera tested in Germany, 0.4% of US military populations, 0.6% of Florida residents and 68% of Oklahoma residents. As of July 2007, only four human infections with marine mammal *Brucella* have been reported. One infection occurred in a researcher exposed in the laboratory. Two patients with community-acquired neurobrucellosis 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 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 spinal osteomyelitis. 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.

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Brucellosis is rarely fatal if treated; in untreated persons, estimates of the case fatality rate vary from less than 2% to 5%. Deaths are usually caused by endocarditis or meningitis.

Infections in Animals

Species Affected

Most species of *Brucella* are maintained in a limited number of reservoir hosts. Maintenance hosts for *Brucella abortus* include cattle, bison (*Bison* spp.) water buffalo (*Bubalus bubalus*), African buffalo (*Syncerus caffer*), elk and camels. A feral pig population was recently reported to maintain *B. abortus* in the U.S. Sheep and goats are the reservoir hosts for *B. melitensis*. Sheep are also the maintenance hosts for *B. ovis*. In addition, *B. ovis* occurs in farmed red deer (*Odocoileus virginianus*) in New Zealand. *B. canis* is maintained in dogs and *B. neotomae* in rodents. *B. suis* contains more diverse isolates than other *Brucella* species, and these isolates have broader host specificity. *B. suis* biovars 1, 2 and 3 affect swine. Biovars 1 and 3 are found in both domesticated pigs (*Sus scrofa domesticus*) and wild or feral pigs. Biovar 2 currently occurs mainly in wild boar (*Sus scrofa scrofa*) and European hares (*Lepus capensis*); however, this biovar can be transmitted from these reservoirs to domesticated pigs, and spreads readily in these herds. Biovar 4 is maintained in caribou and reindeer (*Rangifer tarandus* and its various subspecies). Biovar 5 is found in small rodents. Marine *Brucella* species have been found by culture or serology in many pinniped and cetacean species including seals, sea lions, walruses, porpoises, dolphins, whales and a European otter.

Other species can become accidental hosts, particularly after close contact. . *B. abortus*, *B. melitensis* and *B. suis* infections are reported occasionally in many species including horses, cattle, sheep, goats, camels, pigs, moose, chamois, alpine ibex, raccoons, opossums, dogs, coyotes, foxes and wolves. 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. In contrast, *B. ovis* and *B. canis* seem to be relatively host-specific. Experimental *B. ovis* infections have been reported in goats and cattle, but there is no evidence that these species are infected in nature. Dogs are the only species known to be naturally infected with *B. canis*, although antibodies to this organism have been found in other carnivores. Experimental *B. canis* infections can be established in domesticated livestock and chimpanzees; however, these species are considered highly resistant to natural exposure.

Incubation Period

The incubation period varies with the species and stage of gestation at infection. In cattle, reproductive losses typically occur during the second half of the pregnancy; thus, the incubation period is longer when animals are infected early in gestation. In this species, abortions and stillbirths usually occur two weeks to five months after infection. In pigs, abortions can occur at any time during gestation. In dogs, abortions are most common at approximately 7 to 9 weeks of gestation, but early embryonic deaths have also been reported after 2 to 3 weeks.

Clinical Signs

Bovine brucellosis (B. abortus)

In cattle, *B. abortus* causes abortions, stillbirths and weak calves; abortions usually occur during the second half of gestation. The placenta may be retained and lactation may be decreased. After the first abortion, subsequent pregnancies are generally normal; however, cows may shed the organism in milk and uterine discharges. Epididymitis, seminal vesiculitis, orchitis and testicular abscesses are sometimes seen in bulls. Infertility occurs occasionally in both sexes, due to metritis or orchitis/epididymitis. Hygromas, particularly on the leg joints, are a common symptom in some tropical countries. Arthritis can develop after long-term infections. Systemic signs do not usually occur in uncomplicated infections, and deaths are rare except in the fetus or newborn. Infections in nonpregnant females are usually asymptomatic.

Similar symptoms occur in other ruminants including camels, bison and water buffalo; however, experimentally infected moose develop more serious disease and die rapidly.

Ovine and caprine brucellosis (B. melitensis)

B. melitensis mainly causes abortions, stillbirths and the birth of weak offspring. Animals that abort may retain the placenta. Sheep and goats usually abort only once, but reinvasion of the uterus and shedding of organisms can occur during subsequent pregnancies Milk yield is significantly reduced in animals that abort, as well as in animals whose udder becomes infected after a normal birth. However, clinical signs of mastitis are uncommon. Acute orchitis and epididymitis can occur in males, and may result in infertility. Arthritis is seen occasionally in both sexes. Many non-pregnant sheep and goats remain asymptomatic.

Ovine epididymitis (B. ovis)

B. ovis affects sheep but not goats. This organism can cause epididymitis, orchitis and impaired fertility in rams. Initially, only poor quality semen may be seen; later, lesions may be palpable in the epididymis and scrotum. Epididymitis may be unilateral or, occasionally, bilateral. The testes may atrophy. Some rams shed *B. ovis* for long periods without clinically apparent lesions. Abortions, placentitis and perinatal mortality can be seen in ewes but are uncommon. Systemic signs are rare. *B. ovis* can also cause poor semen quality in red deer stags, but abortions have not been reported in hinds.

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Porcine and rangiferine brucellosis (B. suis)

In pigs, the most common symptoms are abortion, which can occur at any time during gestation, and weak or stillborn piglets. Vaginal discharge is often minimal and abortions may be mistaken for infertility. Occasionally, some sows develop metritis. Temporary or permanent orchitis can be seen in boars. Boars can also shed *B. suis* asymptomatically in the semen; sterility may be the only sign of infection. Swollen joints and tendon sheaths, accompanied by lameness and incoordination, can occur in both sexes. Less common signs include posterior paralysis, spondylitis and abscesses in various organs. Although some pigs recover, others remain permanently infected. Fertility can be permanently impaired, particularly in boars. Some animals remain asymptomatic.

In hares, *B. suis* biovar 2 infection is characterized by nodules in the internal organs, particularly the reproductive organs, as well as the subcutaneous tissues and muscles. The nodules can become purulent. The animal's body condition may be minimally affected.

In caribou and reindeer, *B. suis* biovar 4 can cause abortion and retained placenta. Metritis and mastitis can also occur. Males may develop orchitis. Lameness can occur in both sexes from arthritis, bursitis, tenosynovitis and/ or hygromas. Subcutaneous abscesses are also seen.

Canine brucellosis (B. canis)

B. canis can cause abortions and stillbirths in pregnant dogs. Most abortions occur late, particularly during the seventh to ninth week of gestation. Abortions are usually followed by a mucoid, serosanguinous or gray-green vaginal discharge that persists for up to six weeks. Early embryonic deaths and resorption have been reported a few weeks after mating, and may be mistaken for failure to conceive. Some pups are born live but weak; most die soon after birth. Other congenitally infected pups can be born normal and later develop brucellosis. Clinical signs occur during subsequent pregnancies in some dogs, but not in others. Epididymitis, scrotal edema, orchitis and poor sperm quality may be seen in males. Scrotal dermatitis can occur due to self-trauma. Unilateral or bilateral testicular atrophy can be seen in chronic infections, and some males become infertile.

Lymphadenitis is common in infected dogs. Lethargy or fatigue, exercise intolerance, decreased appetite, weight loss and behavioral abnormalities (loss of alertness, poor performance of tasks) are occasionally reported; however, most affected dogs do not appear seriously ill. Occasionally, discospondylitis of the thoracic and/or lumbar vertebrae can cause stiffness, lameness or back pain. Uveitis, endophthalmitis, polygranulomatous dermatitis, endocarditis and meningoencephalitis have also been reported. Fever is uncommon, and deaths are rare except in the fetus or newborn. Many infected dogs remain asymptomatic.

Brucellosis in horses

In horses, *B. abortus* and occasionally *B. suis* can cause inflammation of the supraspinous or supra-atlantal bursa; these syndromes are known, respectively, as fistulous withers or poll evil. The bursal sac becomes distended by a clear, viscous, straw-colored exudate and develops a thickened wall. It can rupture, leading to secondary inflammation. In chronic cases, nearby ligaments and the dorsal vertebral spines may become necrotic. *Brucella*-associated abortions are rare in horses.

Brucellosis in marine mammals

There is little information on the effects of brucellosis in marine mammals. Reproductive disease is difficult to assess in wild animals, but *Brucella* has been isolated from the reproductive organs of some marine 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. Other signs of *Brucella*-associated systemic disease have been seen 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. It 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.

Communicability

Brucellosis is a communicable disease in animals. Large numbers of bacteria are found in aborted fetuses, fetal fluids and membranes, as well as vaginal discharges and milk. Other secretions and excretions including semen, urine and hygroma fluids can also contain organisms. Bacteria have been reported in the feces of some animals including a harbor seal. Infectious bacteria are also found in the bursa of horses with poll evil or fistulous withers. Some animals can shed *Brucella* long-term or lifelong.

Post Mortem Lesions

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Brucella abortus, B. melitensis and B. suis

Some aborted fetuses appear normal; others are autolyzed or have variable amounts of subcutaneous edema and bloodstained fluid in the body cavities. In ruminant

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fetuses, the spleen and/or liver may be enlarged, and the lungs may exhibit pneumonia and fibrous pleuritis. Abortions caused by *Brucella* spp. are typically accompanied by placentitis. The cotyledons may be red, yellow, normal or necrotic. In cattle and small ruminants, the intercotyledonary region is typically leathery, with a wet appearance and focal thickening. There may be exudate on the surface.

In adults, granulomatous to purulent lesions may be found in the male and female reproductive tract, mammary gland, supramammary lymph nodes, other lymphoid tissues, bones, joints and other tissues and organs. Mild to severe endometritis may be seen after an abortion, and males can have unilateral or bilateral epididymitis and/or orchitis. In *B. abortus*-infected cattle, hygromas may be found on the knees, stifles, hock, angle of the haunch, and between the nuchal ligament and the primary thoracic spines.

In hares, *B. suis* biovar 2 infections are associated with nodules of varying sizes in internal organs, particularly the reproductive organs but also the spleen, liver, lung and most other organs. The skin and subcutaneous tissues can also be affected. These nodules often become purulent. Despite the nodules, the hare's body condition may be good.

Brucella ovis

In rams infected with *B. ovis*, lesions are usually limited to epididymitis and orchitis. Epididymal enlargement can be unilateral or bilateral, and the tail is affected more often than the head or body. Fibrous atrophy can occur in the testis. The tunica vaginalis is often thickened and fibrous, and can have extensive adhesions. Although placentitis is uncommon, it is occasionally seen in infected ewes.

Brucella canis

Aborted puppies are often partially autolyzed and have evidence of generalized bacterial infection. Fetal lesions can include subcutaneous edema, subcutaneous congestion and hemorrhages in the abdominal region, serosanguinous peritoneal fluid, and degenerative lesions in the liver, spleen, kidneys and intestines.

The lymph nodes are often enlarged in affected adults. The retropharyngeal and inguinal lymph nodes are often involved, but generalized lymphadenitis also occurs. The spleen is frequently enlarged, and may be firm and nodular. Hepatomegaly may also be seen. Scrotal edema, scrotal dermatitis, epididymitis, orchitis, prostatitis, testicular atrophy and fibrosis occur in some infected males, and metritis and vaginal discharge may be seen in females. Less commonly reported lesions include discospondylitis, meningitis, focal non-suppurative encephalitis, osteomyelitis, uveitis, and abscesses in various internal organs.

Brucella in marine mammals

In marine mammals, brucellosis has been linked to lesions in a few animals. Reported lesions include meningoencephalitis, subcutaneous abscesses, placentitis/abortion, epididymitis, chronic purulent or granulomatous orchitis, 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 dolphins with meningoencephalitis, the lesions were described as severe, chronic, widespread, nonsuppurative meningitis which was most severe in the brainstem. The meningitis was accompanied by periventricular encephalitis. *Brucella* has also been recovered from apparently normal tissues and animals with no lesions.

Diagnostic Tests

Brucellosis can be diagnosed by culture, serology or other tests.

Microscopic examination

Microscopic examination of smears stained with the Stamp's modification of the Ziehl-Neelsen method can be used for a presumptive diagnosis. Organisms may be found in abortion products, vaginal discharges, milk, semen or various tissues. *Brucell*a species are not truly acid-fast, but they are resistant to decolorization by weak acids, and stain red against a blue background. Brucellae are coccobacilli or short rods, usually arranged singly but sometimes in pairs or small groups. This test is not definitive. Other organisms such as *Chlamydophila abortus* and *Coxiella burnetii* can resemble *Brucella*. Direct examination may not detect the small numbers of organisms present in milk and dairy products.

Culture

Brucella species can be recovered from numerous tissues and secretions, particularly fetal membranes, vaginal secretions, milk (or udder secretions in nonlactating cows), semen, arthritis or hygroma fluids, and the stomach contents, spleen and lung from aborted fetuses. Blood cultures are often used to detect *B. canis* in dogs. In this species, bacteremia (which may be intermittent) can persist for up to five years and possibly longer. Oral, nasal, tracheal, vaginal and anal swabs, as well as feces, can be submitted for culture from marine mammals.

At necropsy, bacteria can be isolated from a variety of organs including lymph nodes, spleen, uterus, udder, testis, epididymis, joint exudate, abscesses and other affected tissues. In ruminants with suspected *B. abortus* or *B. melitensis* infections, the spleen, mammary and genital lymph nodes, udder and late pregnant or early post-parturient uterus are the most reliable samples to collect. The preferred tissues to collect in rams suspected of *B. ovis* infection are the epididymis, seminal vesicles, ampullae and inguinal lymph nodes. In dogs, recommended biopsy or necropsy samples include lymph nodes, prostate, epididymis, testis, uterus, spleen, liver and bone marrow.

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The lymph nodes and spleen are most likely to be positive in non-bacteremic dogs.

Brucella spp. can be isolated on a variety of plain media, or selective media such as Farrell's medium or Thayer-Martin's modified medium. Enrichment techniques can also be used. Colony morphology varies with the species. Colonies of smooth forms (*B. abortus*, *B. suis*, *B. melitensis* and marine mammal *Brucella*) are round with smooth margins. When the plates are viewed in daylight through a transparent medium, these colonies are translucent and a pale honey color. From above, they are convex and pearly white. *B. ovis* and *B. canis* are rough (R) forms. The colonies are round, shiny and convex, but their rough nature can be seen by examining the colony with oblique illumination. Most *Brucella* species form colonies within a few days, but isolates from seals grow slowly and may take 7-10 days to become visible on selective media. *Brucella* isolates can be identified to the species and biovar level by phage typing and cultural, biochemical and serological characteristics. Care should be taken during identification, as marine mammal isolates are sometimes misidentified initially as terrestrial strains. Genetic techniques can also be used for biotyping. The vaccine strains (*B. abortus* strains S19 and RB51, and *B. melitensis* Rev-1) can be distinguished from field strains by their growth characteristics and sensitivity to antibiotics and other additives.

Animal inoculation is rarely used to isolate *Brucella*, but may be necessary if other techniques fail. Guinea pigs or mice can be used.

Serology

Brucellosis is often diagnosed by serology. Serological tests are not completely specific and cannot always distinguish reactions due to *B. melitensis* from cross-reactions to other bacteria, particularly *Yersinia enterocolitica* O:9.

In cattle, sheep and goats, serology can be used for a presumptive diagnosis of brucellosis, or to screen herds. Serological tests commonly used to test individual cattle or herds include the buffered *Brucella* antigen tests (rose bengal test and buffered plate agglutination test), complement fixation, indirect or competitive enzyme-linked immunosorbent assays (ELISAs) and the fluorescence polarization assay. Rivanol precipitation, acidified antigen procedures and the serum agglutination test (tube or microtiter test) are also available. Supplemental tests such as complement fixation or rivanol precipitation are often used to clarify the results from plate or card agglutination tests. ELISAs or the *Brucella* milk ring test (BRT) can be used to screen herds by detecting antibodies in milk. In vaccinated cattle, the native hapten -based gel precipitation tests (gel diffusion or radial immunodiffusion tests) are sometimes used to distinguish vaccination from infection. In sheep and goats, *B. melitensis* can be diagnosed with the buffered *Brucella* antigen tests, complement fixation or ELISAs. Native hapten -based gel precipitation tests are also used in vaccinated sheep and goats. The bulk milk ring test is not used in small ruminants. Serological tests used to detect *B. ovis* include ELISAs, agar gel immunodiffusion (AGID) and complement fixation. Other tests including hemagglutination inhibition and indirect agglutination have also been described.

Serological tests used to detect *B. canis* in dogs include rapid slide agglutination (card or RSAT) tests, tube agglutination, an indirect fluorescent antibody (IFA) test, AGID and ELISA.

In swine, serology is generally considered to be more reliable for identifying infected herds than individual pigs. Serological tests used in swine include ELISAs, the buffered *Brucella* antigen tests and complement fixation. A fluorescence polarization assay has been developed. Supplemental serological tests used in cattle may also be used in swine.

The serological tests used in marine mammals have been adapted from livestock *Brucella* tests. They include the buffered *Brucella* antigen tests, serum agglutination tests, complement fixation, AGID, ELISAs and rivanol test. In general, these tests have not yet been validated for marine mammals; threshold values have not been established and can vary between laboratories.

Other tests

Immunostaining techniques are sometimes used to detect *Brucella* antigens in tissue samples. A brucellin allergic skin test can be used to test pigs for *B. suis*, or unvaccinated small ruminants and cattle for *B. melitensis* or *B. abortus*, respectively. Polymerase chain reaction (PCR) techniques are also available for most species.

Treatment

There is no practical treatment for infected cattle or pigs, but long-term antibiotic treatment is sometimes successful in infected dogs. Some dogs relapse after treatment. Antibiotic treatment has also been used successfully in some valuable rams, but it is usually not economically feasible. Fertility may remain low even if the organism is eliminated. In horses with fistulous withers or poll evil, the infected bursa may need to be surgically removed.

Prevention

Brucellosis is usually introduced into a herd or kennel in an infected animal, but it can also enter in semen. Herd additions should come from brucellosis-free areas or accredited herds. *B. ovis*-free accredited rams may be available in some areas. Animals from other sources should be isolated and tested before adding them to the herd. Domesticated animals should always be kept from contact with wild animal reservoirs. Commercial *B. abortus* and *B. melitensis* vaccines are available for cattle, sheep and goats. Vaccination can interfere with serological tests; this is

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minimized when only young animals are vaccinated. Vaccination for *B. ovis* is practiced in New Zealand and some other countries, but not in the U.S. Successful vaccines have been difficult to develop for pigs; this species is generally not vaccinated except in China. No vaccines are made for dogs. Vaccines have not been successful in preventing fistulous withers or poll evil in horses.

B. abortus, *B. melitensis* and *B. suis* can be eradicated from a herd by test-and-removal procedures, or by depopulation. Some swine programs are designed to retain desirable genetic characteristics in the herd. Good management can reduce the incidence of infection in an infected herd. Whenever possible, animals should give birth in individual pens. Transmission is reduced by immediate disposal of the placenta, contaminated bedding and other infectious material, followed by thorough cleaning and disinfection. The prevalence of *B. ovis* can be decreased by examining rams before the breeding season and culling rams with palpable abnormalities. However, palpable lesions are not found in all infected rams, and laboratory testing of rams should also be considered. Test-and-removal methods directed at rams can eradicate this organism from a flock. *B.-ovis*-free infections in ewes are generally prevented by controlling infections in rams. Infections in other species are generally prevented by controlling *Brucella* species in their maintenance hosts.

Nationwide eradication programs for *B. abortus*, *B. melitensis* and *B. suis* include quarantines of infected herds, vaccination, test-and-slaughter and/or depopulation techniques, cleaning and disinfection of infected farms, and various forms of surveillance and tracebacks. *B. ovis* has been eradicated from sheep in the Falkland Islands by test-and-removal methods directed at rams. In areas where a *Brucella* species is not endemic, infected herds are usually quarantined and the animals are euthanized. In the U.S., *B. suis* has been eradicated from commercial swine, and *B. abortus* has nearly been eradicated from domesticated ruminants. Various control methods are being directed at wild animal reservoirs including wild bison and elk herds in the Greater Yellowstone Area, and wild and feral swine.

Canine brucellosis can be controlled similarly to livestock brucellosis, by sanitation and the removal of infected dogs. Housing in individual cages reduces the spread of the organism. Repeated testing and the removal of seropositive or culture-positive animals, combined with quarantine and testing of newly added dogs, have been used to eradicate brucellosis from some kennels. Long-term antibiotic therapy may be tried in some infected dogs. Neutering can be used as an additional control measure.

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 practiced with infected animals. Some authors suggest that centers involved in marine mammal rehabilitation should routinely screen animals for *Brucella*.

Morbidity and Mortality

B. abortus, *B. melitensis* and *B. suis* are associated with a high morbidity rate in naïve herds, and a much lower morbidity rate in chronically infected herds. In naive cattle, *B. abortus* spreads rapidly, and 30% to 80% of the herd may abort. In herds where this organism has become endemic, only sporadic symptoms occur and cows may abort their first pregnancies. A similar pattern is seen with *B. melitensis*-infected sheep and goats. Likewise, when *B. suis* is first introduced into a herd, there may be a significant increase in returns to service, abortions and stillbirths, weak piglets, lameness/ arthritis, posterior paralysis and other signs. The pre-weaning mortality rate usually increases. However, in endemic swine herds, brucellosis may appear as non-specific infertility, a slightly reduced farrowing rate, and irregular estrus cycles. In domesticated pigs, the abortion rate from *B. suis* varies widely, from 0% to 80%. Fertility can be permanently impaired after infection with some species of *Brucella*. Deaths are rare in adult animals of most species; however, *B. abortus* can be lethal in experimentally infected moose, and possibly in bighorn sheep.

B. ovis has little effect on sperm quality in some individual animals, but causes severe decreases in sperm motility, concentration and morphology in others. Approximately 30-50% of all infected rams have palpable lesions of the epididymis. Estimates of the abortion rate vary. Some sources report that *B. ovis* causes abortion and perinatal lamb mortality rates of 1–2%, while others suggest that these outcomes are rare. Limited experimental studies have reported abortion rates from 0% to 8%. Abortions and increased perinatal mortality have not been reported in red deer hinds.

B. canis spreads rapidly in confined populations, particularly during breeding or when abortions occur. Although death is rare except in the fetus and neonate, significant reproductive losses can be seen, particularly in breeding kennels. Up to 75% fewer puppies may be weaned from affected kennels.

The morbidity and mortality rates for brucellosis in marine mammals are unknown.

Internet Resources

Centers for Disease Control and Prevention (CDC). Brucellosis. http://www.cdc.gov/brucellosis/

European Commission. Brucellosis in Sheep and Goats (*Brucella melitensis*). http://ec.europa.**eu**/food/fs/sc/scah/out59_en.pdf

Food and Agriculture Organization of the United Nations. Manual for the Recognition of Exotic Diseases of Livestock, A Reference Guide for Animal Health Staff. http://www.spc.int/rahs/**

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Medical Microbiology
http://www.ncbi.nlm.nih.gov/books/NBK7627/

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

World Organization for Animal Health (OIE)
http://www.oie.int

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals
http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/

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