Porcine and Rangiferine Brucellosis: Brucella suis

Enzootic Abortion, Contagious Abortion, Undulant Fever,

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IOWA STATE UNIVERSITY®

College of Veterinary Medicine Iowa State University Ames, Iowa 50011 Phone: 515.294.7189 Fax: 515.294.8259 cfsph@iastate.edu www.cfsph.iastate.edu



INSTITUTE FOR INTERNATIONAL COOPERATION IN ANIMAL BIOLOGICS

Iowa State University College of Veterinary Medicine www.cfsph.iastate.edu/IICAB/

Importance

Porcine brucellosis, caused by the bacterium *Brucella suis*, is an economically important cause of reproductive loses in pigs. This organism can be maintained in wild and feral swine, complicating eradication efforts in domesticated pigs. One variant, found only in Europe, is also maintained in hares. Some variants of *B. suis* mainly affect caribou, reindeer or rodents, and are not important in pigs.

B. suis is zoonotic. In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs. Most cases are the result of occupational exposure to infected animals. *B. suis* has also been weaponized and could be used in a bioterrorist attack.

Etiology

In pigs, brucellosis is mainly caused by *Brucella suis*, a Gram-negative coccobacillus or short rod. This organism is a facultative intracellular pathogen. Other *Brucella* species rarely found in pigs include *Brucella abortus* and *B. melitensis* (For information on *B. abortus* or *B. melitensis*, see the factsheets titled "Bovine Brucellosis" and "Ovine and Caprine Brucellosis," respectively.)

B. suis contains more diverse isolates than other *Brucella* species, and these isolates have broader host specificity. Five *B. suis* biovars have been identified. 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, although is genetically very closely related to biovar 1. Biovar 5 occurs in rodents in the former USSR. Biovar 5 is distinct from other *B. suis* biovars, and may be more closely related to marine mammal *Brucella* isolates.

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.

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. *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 4 can also infect moose, cattle, Arctic foxes and wolves. Biovar 5 is found in small rodents. Various *B. suis* biovars have been reported occasionally in cattle, small ruminants, horses, dogs and other spillover hosts.

Biovars 1, 3 and 4 are important human pathogens. Biovar 2 is zoonotic, but has been reported very rarely from humans.

Geographic Distribution

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, this organism 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. Cases have occasionally been reported from some African nations, including Uganda and Cote d'Ivoire.

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

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including Siberia, Canada and Alaska. Biovar 5 (murine brucellosis) occurs in the former USSR.

Transmission

In pigs, *B. suis* occurs in the fetus, placenta, fetal fluids and vaginal discharges after an abortion or stillbirth. Pigs usually become infected when they ingest feed contaminated by birth or abortion products, or eat aborted fetuses and membranes. Venereal transmission is also common in swine. *B. suis* is shed in semen; both symptomatic and asymptomatic boars can excrete bacteria. Transmission could occur by inhalation, through the conjunctiva or via broken skin, but these routes seem to be of minimal epidemiological significance in pigs. Piglets can be infected during nursing, but most seem to reach weaning age without becoming infected. In swine, the initial bacteremia can persist for up to 90 days. Some animals recover from the infection, while others remain permanently infected.

B. suis biovar 4 (rangiferine brucellosis) may be transmitted in reindeer and caribou by contact with aborted fetuses and fetal membranes. The importance of venereal transmission or ingestion during nursing is unknown.

Other species can be infected with *B. suis* after contact with infected pigs or other maintenance hosts. Humans usually become infected by the contamination of mucous membranes and abraded skin, but they can also be infected by ingesting organisms. Because porcine isolates of *B. suis* can colonize the bovine udder, unpasteurized milk can be a source of infection for people. Biovar 4 (rangiferine brucellosis) is generally transmitted to humans by direct contact, through unpasteurized milk products and in bone marrow, which is a regional delicacy.

B. suis can be spread on fomites, particularly 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* species 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.

Incubation Period

The period between infection and the development of reproductive signs is variable; in pigs, abortions can occur at any time during gestation.

Clinical Signs

In pigs, the most common symptoms are abortion, which can occur at any time during gestation, and weak or stillborn piglets. Weak piglets may die before weaning. Vaginal discharge is often minimal in pigs that abort, and abortions may be mistaken for infertility. Occasionally, some sows develop metritis. *B. suis* can also cause epididymitis and orchitis in boars. In boars, an initial fever may be followed by testicular pain, reluctance to mate and

abnormalities in the semen. Abscesses and swelling are sometimes seen, and the testes may become sclerotic and atrophy during the final stages of disease. The lesions are often unilateral. Boars can also excrete *B. suis* asymptomatically in the semen, and 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, metritis and abscess formation 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.

Horses exposed to infected pigs can also be infected, although this occurs rarely. In horses, *B. suis* usually causes inflammation of the supraspinous or supra-atlantal bursa; this syndrome is 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.

In caribou and reindeer, *B. suis* biovar 4 can cause abortion and retained placenta in females. Metritis can occur, and may be accompanied by a bloodstained discharge. Mastitis is also seen. Orchitis can occur in males. Lameness may be seen in both sexes, as a result of arthritis, bursitis, tenosynovitis and/ or hygromas. Subcutaneous abscesses also occur. Moose seem to be infected rarely with biovar 4, and information on the clinical signs in this species is limited. Septicemia, anorexia, fever and depression were observed in an experimentally infected moose. Carpal pathology and osteomyelitis occurred in an emaciated, debilitated moose found infected in the wild; other tissues from this animal were not submitted for examination.

Post Mortem Lesions di Click to view images

At necropsy, abscesses, other purulent or inflammatory lesions, or calcified foci may be seen in the testes and male accessory sex organs, particularly the epididymis and seminal vesicles .In boars, lesions tend to be unilateral. After an abortion, the placenta may be edematous and hyperemic, and the fetus may have hemorrhagic fluid in the peritoneal space and subcutaneous tissues. The placenta may be retained. Metritis sometimes occurs in sows, and nodules and abscesses may be found in both the gravid and non-gravid uterus. Abscesses or other purulent lesions can also be found in non-reproductive organs, particularly the lymph nodes, spleen, liver, kidneys, joint capsules, tendon sheaths, bones, mammary gland, urinary bladder and occasionally the brain. Nodular splenitis, arthritis, bursitis and osteomyelitis of the vertebral bodies have also been reported.

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. The body condition may be good.

In horses, there may be inflammation of the supraspinous or supra-atlantal bursa. The bursal sac is typically distended, has a thickened wall, and contains a clear, viscous, straw-colored exudate. Infected bursae can rupture, leading to secondary inflammation. In chronic cases, nearby ligaments and the dorsal vertebral spines can become necrotic.

Morbidity and Mortality

B. suis has been eradicated from domesticated swine in some countries, including the U.S. However, this infection still occurs in wild and feral pigs in some areas, and can spread to domesticated herds. In studies conducted in South Carolina, the prevalence of infection in wild and feral swine ranged from 14% to 44%; in one case, the percentage of infected pigs in a location also varied over time, from 28% in 1976 to 18% in 1992 and 44% in 1999. In another study, 6% of the wild and feral pigs sampled in eleven southeastern states and Hawaii were seropositive. Similarly, biovar 2 is thought to be widespread in wild boar in Europe, although infections are not always associated with gross lesions. Domesticated pigs reared outdoors are at highest risk of infection by biovar 2.

The morbidity rate varies with the length of time *B. suis* has been in the herd. When this organism 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. In endemic 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%. Natural resistance to *Brucella suis* exists among swine, and pigs can be bred to increase their resistance to infection. Deaths are rare except in the fetus and newborn.

Diagnosis

Clinical

Porcine brucellosis can be difficult to diagnose and is usually recognized as a herd problem rather than a disease of individual swine. Porcine brucellosis should be suspected when reproductive failure in sows, orchitis in boars and lameness, arthritis or paralysis occur together in a herd. In herds where *B. suis* has become endemic, the symptoms can be subtle and this classical presentation may not be seen.

Differential diagnosis

Other diseases causing abortion, orchitis, arthritis, posterior paralysis and lameness should be considered. In pigs, the differential diagnosis for abortion includes a number of other diseases including Aujeszky's disease, leptospirosis, erysipelas, salmonellosis, streptococcidiosis, classical swine fever and porcine parvovirus infection.

Laboratory tests

Microscopic examination of smears stained with the Stamp's modification of the Ziehl-Neelsen method can be useful for a presumptive diagnosis. *Brucella* 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 can resemble *Brucella*.

In swine, serology is generally considered to be more reliable for identifying infected herds than individual pigs. Serological tests are not completely specific and cannot always distinguish reactions due to B. suis from crossreactions to other bacteria, particularly Yersinia enterocolitica O:9. Low agglutinin titers can be seen in most herds, even if they are uninfected. A few infected pigs, particularly weaners, do not have a detectable titer. Serological tests used in swine include indirect or competitive enzyme-linked immunosorbent assays (ELISAs), the buffered Brucella antigen tests (rose bengal test and buffered plate agglutination test) and complement fixation. A fluorescence polarization assay has been developed. Supplemental serological tests used in cattle may also be used in swine.

A brucellin allergic skin test is widely used to identify infected herds in some countries, but is not generally used in the U.S. This test is performed by injecting the allergen intradermally at the base of the ear.

A definitive diagnosis can be made if *B. suis* is cultured from an animal. *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. *Brucella* colonies usually become visible after two days growth. *B. suis* is a smooth (S) form, like *B. abortus*. After four days of incubation, the colonies are round and approximately 1-2 mm in diameter with smooth margins. When the plates are viewed in daylight through a transparent medium, the colonies are translucent and a pale honey color. From above, they are convex and pearly white. *B. suis* can be identified to the species and biovar level by phage typing and cultural, biochemical and serological characteristics.

Polymerase chain reaction (PCR) techniques are available in some laboratories.

B. suis biovar 4 can be identified using the same culture techniques as *B. suis* biovars 1, 2 and 3 in pigs. Serological tests that have been used in reindeer and caribou include the tube agglutination test and complement fixation.

Samples to Collect

B. suis biovars 1, 3 and 4 are highly pathogenic for humans; samples should be collected and handled with all appropriate precautions.

A variety of samples can be collected for culture and microscopic examination. Vaginal swabs, semen or blood samples can be submitted from live animals. Testicles can be submitted after castration. The placenta or aborted/ stillborn fetuses can also be cultured. At necropsy, *B. suis* can be isolated from lymph nodes and various organs including the spleen, liver and reproductive organs (uterus, testis, epididymis, seminal vesicles, bulbourethral glands).

Serum should be collected for serology.

Recommended actions if brucellosis is suspected

Notification of authorities

B. suis infections are reportable in the U.S. All suspected or confirmed cases should be reported immediately to state or federal authorities.

Federal: Area Veterinarians in Charge (AVIC): <u>www.aphis.usda.gov/animal_health/area_offices/</u> State Veterinarians: www.usaha.org/Portals/6/StateAnimalHealthOfficials.pdf

Control

B suis is usually introduced into a herd in an infected animal. Semen can also be a source of infection. Herd additions for uninfected herds should come from brucellosis-free states, countries or accredited herds whenever possible. Animals from other sources should be isolated and tested before adding them to the herd. *B. suis* also exists in wild and feral swine in some countries, including the U.S. Domesticated swine should always be kept from contact with these animals. In the U.S., there are programs to control the spread of brucellosis in wild and feral swine populations.

Brucellosis can be eradicated from infected herds by depopulation or test and removal methods; some programs are designed to retain desirable genetic characteristics in the herd. Transmission is also reduced by immediate disposal of the placenta, contaminated bedding and other infectious material, followed by thorough cleaning and disinfection. Many countries have eradication programs for *B suis*. Eradication can be accomplished by quarantine of infected herds, test-and-slaughter or depopulation, various forms of surveillance and tracebacks. In the U.S., *B. suis* has been eradicated from commercial swine, and any infected herds found are quarantined and depopulated. Infections in other

species are generally prevented by controlling *B. suis* in its maintenance hosts.

No vaccine currently exists for *B. suis* in most countries. A *B. suis* strain 2 vaccine is used to immunize pigs in China, but has not gained acceptance elsewhere.

Brucella species are readily killed by most commonly available disinfectants including hypochlorite solutions, isopropanol, iodophores, 70% ethanol. 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

B. suis biovars 1, 3 and 4 are important human pathogens. Human infections are usually the result of occupational exposure in laboratory workers, abattoir workers, farmers, herders, veterinarians and others who contact infected animals or tissues. Porcine isolates of *B. suis* biovar 1 can also colonize the bovine udder; unpasteurized milk products are an important source of infection in some countries. In Queensland, Australia, a number of infections have been reported in hunters who handled tissues from feral pigs. Human infections with biovar 4 are mainly found among herders of diseased reindeer or caribou. Biovar 2 has been reported from humans, and biovar 5 has not been reported from humans to date.

Some *Brucella* infections are asymptomatic in humans. 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. 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

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personality changes, meningitis, uveitis and optic neuritis), anemia, internal abscesses, nephritis, endocarditis and dermatitis can also occur. 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/brucellosis/

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/**

Public Health Agency of Canada. Material Safety Data Sheets

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

The Merck Manual <u>http://www.merck.com/pubs/mmanual/</u>

The Merck Veterinary Manual <u>http://www.merckvetmanual.com/mvm/index.jsp</u>

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

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

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