Network for wildlife health surveillance in Europe

Diagnosis Card

Brucellosis

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Etiology

Gram-negative, facultative, intracellular bacteria. Eleven species are recognized within the genus *Brucella*, namely: *B. abortus*, *B. melitensis*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*, *B. ceti*, *B. pinnipedialis*, *B. microti*, *B. inopinata* and *B. papionis*. Zoonotic disease.

Affected species (wildlife, domestic animals, humans)

Terrestrial mammals:

Brucella infections have been documented worldwide in a great variety of terrestrial species. Maintenance hosts for *B. abortus* include cattle, American and European bison (*Bison bison* and *B. bonasus*, respectively), water buffalo (*Bubalus bubalis*), African buffalo (*Syncerus caffer*), red deer (*Cervus elaphus*) and one- or two-humped camels.

Domestic sheep and goats are the reservoir hosts for *B. melitensis*.

Sheep are the maintenance hosts for *B. ovis*.

B. canis is maintained in dogs.

B. suis consists of five biovars. Biovars 1 and 3 are found in domesticated, wild or feral pigs. Biovar 2 currently occurs in Europe in wild boar and European hares (*Lepus europaeus*); however, this biovar can be transmitted from these reservoirs to domesticated pigs, and spreads readily in herds. Biovar 4 is maintained in caribou and reindeer (*Rangifer tarandus* and its various subspecies).

Other animal species can become accidental hosts. *B. abortus*, *B. melitensis* and *B. suis* infections are reported occasionally in many species including horses, cattle, yaks, sheep, goats, camelids, pigs, moose (*Alces alces*), chamois (*Rupicapra rupicapra*), alpine ibex (*Capra ibex*), South-american camelids, various African antelope species, raccoons (*Procyon lotor*), opossums (*Didelphis virginiana*), dogs, coyotes, various species of foxes and wolves. In contrast, *B. ovis* and *B. canis* seem to be relatively host-specific. Dogs are the only species known to be naturally infected with *B. canis*, although antibodies to this organism have been found in other carnivores.

Marine mammals:

B. pinnipedialis and *B. ceti* have been found by culture or serology in many pinniped and cetacean species including seals, sea lions, walruses (*Odobenus rosmarus*), porpoises, dolphins, whales and a European otter (*Lutra lutra*).

Other Brucella species:

B. suis biovar 5, *B. neotomae* and *B. microti* have been reported on few occasions in small rodents respectively in the USA, in Russia and in Central Europe. *B. papionis* has been reported once in captive baboons. *B. inopinata* has been reported only in 2 humans. Epidemiological information available on these later species is scarce.

*B. abortus***,** *B. melitensis* and *B. suis* are highly pathogenic for humans.

Epidemiological characteristics and disease course

Initial infection in the reservoir species is often followed by abortion. Infection becomes usually chronic in animals; treatment is rarely undertaken and usually forbidden in domestic species.

Infected animals shed the organisms in uterine discharge following abortion and subsequent parturition, and also in colostrum and milk. Congenital (in utero) or perinatal infections may also occur. Semen may also be contaminated in infected males and sexual transmission is common in domestic species. Spread between herds usually occurs through contact between neighbouring herds or by the introduction of asymptomatic chronically-infected animals. Human brucellosis is mainly an occupational disease of professionals working with infected animals or their tissues (e.g. veterinarians, breeders, inseminators, abattoir or laboratory workers). *Brucella* can also infect consumers of unpasteurized dairy products, and hunters who unknowingly handle infected animals.

Clinical signs

B. abortus and *B. melitensis* cause abortions, stillbirths and weak calves; abortions usually occur during the second half of gestation. The placenta may be retained. After the first abortion, subsequent pregnancies are generally normal. Infertility occurs occasionally in both sexes, due to metritis or orchitis/epididymitis. Hygromas, particularly on the leg joints, are recorded. Infections in non-pregnant females are usually asymptomatic. Similar symptoms occur in other ruminants including camels, bison and water buffalo. *B. melitensis* in chamois and ibex may be responsible of blindness and neurological signs, thick-walled carpal joints and enlargement of the testicle characterized by necrosis and fibrosis. Retained placenta or infertility has not been observed in infected Red deer. Moose are very susceptible to infection with *B. abortus*, and die rapidly.

B. ovis affects sheep but not goats. It can cause epididymitis, orchitis and impaired fertility in rams. Initially, only poor quality semen may be seen. Abortions and perinatal mortality can be seen in ewes but are uncommon. *B. ovis* can also cause poor semen quality in red deer *(Cervus elaphus)*, but abortions have not been reported.

In pigs, the most common signs of *B. suis* infection are abortion, which can occur any time during gestation, weak or stillborn piglets. Vaginal discharge is often minimal and abortions may be mistaken for infertility. Occasionally, some sows develop metritis. Sterility may be the only sign of infection. Swollen joints and tendon sheaths, accompanied by lameness and incoordination, can occur in both sexes. During *B. suis* biovar 2 infection, hares' body condition may be minimally affected, but subcutaneous or deep abscesses (spleen, liver) as well as metritis may be observed. In caribou and reindeer (*Rangifer tarandus*), *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.

In dogs, *B. canis* can cause abortions, during the seventh to ninth week of gestation, and stillbirths in pregnant dogs. Some pups are born live but weak; most die soon after birth. Epididymitis, scrotal edema, orchitis and poor sperm quality may be seen in males. Fever is uncommon, and death is rare except in the newborn. Many infected dogs remain asymptomatic.
Marine mammals' brucellosis has been associated with meningoencephalitis, reported in stranded striped dolphins (*Stenella coeruleoalba*), and with systemic disease, mainly in Atlantic white-sided dolphins (*Lagenorhynchus acutus*). *Brucella*-associated abortions and placentitis were reported in two captive bottlenose dolphins (*Tursiops truncatus*). *Brucella*-associated epididymitis has been reported in porpoises, and orchitis from suspected brucellosis was reported in mink whales (*Balaenoptera acutorostrata*).

Gross lesions

Abortion is associated with necro-hemorrhagic placentitis. Fibrinous pleuritis and pericarditis, and interstitial pneumonia may occur in fetuses. As described for *B. melitensis*, *B. abortus* infection may lead to a mild to moderate interstitial mastitis, resulting in intermittent shedding of the pathogen in the milk. Adult males may develop orchitis, often associated with a seminal vesiculitis and epididymitis. Testicular abscesses are sometimes seen in bulls during infection with *B. abortus* and *B. melitensis.* Hygromas, usually involving leg joints, are a common manifestation of brucellosis.

In hares, *B. suis* biovar 2 infection is characterized by nodules in the internal organs, particularly the reproductive organs, as well as in the subcutaneous tissues and muscles. The nodules can become purulent. Lymphadenitis is common in infected dogs. Uveitis, endophthalmitis, polygranulomatous dermatitis, endocarditis and meningoencephalitis have also been reported.

In marine mammals, systemic disease lesions include hepatic and splenic necrosis, lymphadenitis and mastitis. *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.

Histological lesions

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.

Differential diagnosis

Abortion diseases and causes of orchitis, arthritis and synovitis.

Criteria for diagnosis

A combination of growth characteristics, serological, bacteriological and/or molecular methods is usually needed. Unequivocal diagnosis of *Brucella* infections can be made only by the isolation and identification of *Brucella*, but in situations where bacteriological examination is not practicable, diagnosis may be based on serological methods.

Recommended diagnostic method(s) and preferred samples (incl. recommended amount and appropriate storage)

The diagnostic methods to be used will depend on the objective of the diagnosis (survey, diagnosis confirmation, etc.) and on the available resources (laboratory equipment in particular).The testing strategy (test combination) depends on the epidemiological situation which greatly influences the predictive value of the diagnosis.

Isolation and identification of the agent:

Applicable on all animal species without significant variations in sensitivity and specificity. A variety of samples can be collected for culture and microscopic examination (milk, stomach content, spleen and lung from aborted fetuses, placenta, spleen, head, mammary and genital lymph nodes, semen, testis or epididymis, and arthritis or hygroma fluids). Samples should be kept refrigerated and sent within 48 hours, otherwise they should be stored at -20°C and sent as soon as possible to experienced laboratories.

- Microscopic examination of smears stained with the Stamp's modification of the Ziehl-Neelsen method can be used for a presumptive diagnosis. This method has a low sensitivity and specificity but is easy to perform and cheap.

- Bacteriological culture and isolation on a variety of basal media, or selective media such as Farrell's medium and/ Thayer-Martin's modified medium or selective and translucent culture medium named CITA. This method has a sensitivity ranging between 60 and 85% depending on the starting matrices (high: miscarriage and placenta; low: milk, organs samples and chronic forms), and a specificity of 100%.

- PCR techniques with different characteristics of sensitivity and specificity. It is currently used as a complementary diagnostic method. PCR is fast and may also detect dead *Brucella*.

These techniques need the handling of potentially infectious material and are therefore hazardous. They should be performed in experienced and well-equipped laboratories to minimise occupational exposure.

Serological tests:

Serological tests have not been validated for wildlife. However Rose Bengal test, Complement Fixation Test and the Fluorescence Polarization Assay are generally used for serological diagnosis with the same antigens as for domestic animals. The indirect or competitive enzyme-linked immunosorbent assays (ELISAs) can be used on sera of wildlife for species similar to domestic species (e.g. swine-wild boar, bovine-buffalo/deer, caprine-ibex). The serum samples should be kept refrigerated and sent within 48 hours, otherwise the serum should be stored at -20°C and sent as soon as possible to the laboratory.

APHAEA protocol (for harmonization at large scale)

Bacteriological culture and PCR from target samples. Serological procedures may be used for some wild animals, but each test should be validated in the corresponding animal species. All tests should be performed as prescribed by the OIE Manual (OIE 2014).

Laboratories that can be contacted for diagnostic support

EU/OIE/FAO Brucellosis Reference Laboratory, Anses, Maisons-Alfort, France.

OIE Brucellosis Reference Laboratory, Istituto G. Caporale, Teramo, Italy.

OIE Brucellosis Reference Laboratory, APHA, Weybridge, UK *(*particularly for marine mammals*).*

Recommended literature

Davis DS. 1990. Brucellosis in wildlife. In: Animal brucellosis, Nielsen K, Duncan JR, editors. CRC Press, Florida, 321-324.

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Godfroid J, Garin-Bastuji B, Saegerman C, Blasco JM. 2013. Brucellosis in Terrestrial Wildlife. Rev. sci. tech. Off. int. Epiz. 32(1): 27-42.

Center for Food Security and Public Health (http://www.cfsph.iastate.edu/)

OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. 2014 online version. http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/