Brucella species

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General characteristic

- Small Gram-negative coccobacilli
- Stain red using the modified Ziehl-Neelsen method
- Aerobic and capnophilic(A microorganism that requires or grows best in presence of high concentrations of carbon dioxide).
- Non-motile,
- catalase-positive
- Most isolates are oxidase-positive
- Urease-positive
- Intracellular pathogens
- Target reproductive organs of certain species of animals
- Some species cause undulant fever in humans

Usual habitat

- Brucella have a predilection for both female and male reproductive organs in sexually mature animals and each *Brucella* species tends to infect a particular animal species.
- Infected animals serve as reservoirs of infection, which often persists indefinitely.

Usual habitat

- Organisms shed by infected animals can remain viable in a moist environment for many months.
- However, transmission is usually through direct contact with infected animals or fluids and tissues associated with abortion.

Differentiation of Brucella species

- Brucella species are differentiated by colonial appearance, biochemical tests, specific cultural requirements.
- In addition, agglutination with monospecific sera, susceptibility to bacteriophages and molecular methods are employed for definitive identification.

Differentiation of Brucella species

- On primary isolation, colonies of *B. abortus and B. melitensis* occur in smooth forms and are small, glistening, bluish and translucent after incubation for 3 to 5 days.
- In contrast, primary isolates of *B. ovis* and *B. canis* always occur in rough forms.
- Brucellae are non-haemolytic on blood agar.
- Slide agglutination tests with monospecific antisera are used to detect the presence of important surface antigens, *abortus* antigen A and *melitensis* antigen M.
- The R antigen, a feature of the rough brucellae *B. ovis* and *B. canis*, can be detected by anti-R serum.

Pathogenesis and pathogenicity

- The establishment and outcome of infection with brucellae depend on the number of infecting organisms and their virulence and also on host susceptibility, including age of the host
- Brucellae which lack the major outer-membrane lipopolysaccharide, produce rough colonies and are less virulent than those derived from smooth colonies

Pathogenesis and pathogenicity

- Brucellae persist within macrophages but not within neutrophils.
- Non-opsonized brucellae are taken up through interaction of the O side-chains of LPS with cholesterol-rich phagocyte plasma membrane, termed lipid rafts.

Pathogenesis and pathogenicity

- Once engulfed, brucellae persist within the acidified phagosome, or ‘Brucella-containing vacuole’.
- Inhibition of phagosome–lysosome function is a major mechanism for intracellular survival and an important determinant of bacterial virulence

Pathogenesis and pathogenicity

- While survival within the phagosome occurs, replication of brucellae only takes place once the ‘brucellosome’ is formed.
- This structure is formed through the fusion of the *Brucella*-containing vacuole with the rough endoplasmic reticulum of the host cell.

Pathogenesis and pathogenicity

- In the next phase of infection, virulent brucellae are transported to regional lymph nodes.
- Intermittent bacteremia results in spread and localization in the reproductive organs and associated glands in sexually mature animals.
- Erythritol, a polyhydric alcohol which acts as a growth factor for brucellae, is present in high concentrations in the placentae of cattle, sheep, goats and pigs.

Pathogenesis and pathogenicity

- This growth factor is also found in other organs such as the mammary gland and epididymis, which are targets for brucellae.
- Intracellular replication in trophoblastic cells is strongly influenced by the stage of gestation and increases in late gestation, when the cells actively secrete steroid hormones.

Pathogenesis and pathogenicity

- In chronic brucellosis, organisms may localize in joints or intervertebral discs.
- Brucellae may inhibit or delay the host immune response and this may be responsible in part for the persistent infections seen with this pathogen .

Diagnostic procedures

- The diagnosis of brucellosis depends on serological testing and on the isolation and identification of the infecting *Brucella* species.
- Care should be taken during collection and transportation of specimens, which should be processed in a biohazard cabinet.

Diagnostic procedures

- Specimens for laboratory examination should relate to the specific clinical condition encountered.
- MZN-stained smears from specimens, particularly cotyledons, foetal abomasal contents and uterine discharges, often reveal characteristic MZN-positive coccobacilli.

Diagnostic procedures

- The polymerase chain reaction can be used to detect brucellae in clinical specimens.
- A nutritious medium such as Columbia agar, supplemented with 5% serum and appropriate antimicrobial agents, is used for isolation. Plates are incubated at 37°C in 5 to 10% CO2

Diagnostic procedures

- Serological testing is used for international trade and for identifying infected herds or flocks and individual animals in national eradication schemes .
- Brucellae share antigens with some other Gram-negative bacteria such as *Yersinia enterocolitica* serotype O:9 and consequently cross-reactions can occur in agglutination

Tests used for the diagnosis of bovine brucellosis using milk or serum.

Test	Comments
Brucella milk ring test	Conducted on bulk milk samples for monitoring infections in dairy herds. Sensitive but may not be reliable in large herds
Rose-Bengal plate test	Useful screening test. Antigen suspension is adjusted to pH 3.6, allowing agglutination by lgG1 antibodies. Qualitative test only, positive results require confirmation by CFT or ELISA
Complement-fixation test (CFT)	Widely accepted confirmatory test for individual animals
Indirect ELISA	Reliable screening and confirmatory test
Competitive ELISA (using monoclonal antibodies)	Recently developed test with high specificity; capable of detecting all immunoglobulin classes and can be used to differentiate infected animals from S19-vaccinated cattle
Serum agglutination test (SAT)	A tube agglutination test which lacks specificity and sensitivity; IgG1 antibodies may not be detected, leading to false-negative results
Antiglobulin test	Sensitive test for detecting non-agglutinating antibodies not detected by the SAT

Clinical infections

- Although each *Brucella* species has its own natural host, *B. abortus, B. melitensis* and biotypes of *B. suis* can infect animals other than their preferred hosts

Bovine brucellosis

- Bovine brucellosis, caused by *B. abortus* and formerly worldwide in distribution, has been eradicated or reduced to a low prevalence in many countries through national eradication programmes.
- large numbers of animals may be infected by an aborting cow especially when animals are in close contact indoors.

Bovine brucellosis

- Abortion storms may be encountered in herds with a high percentage of susceptible pregnant cows.
- Abortion usually occurs after the fifth month of gestation and subsequent pregnancies are usually carried to term.

Bovine brucellosis

- Large numbers of brucellae are excreted in uterine discharges for about 2 to 4 weeks following an abortion and at subsequent parturitions, although infected calves appear normal.
- Infection in calves is of limited duration in contrast to cows in which infection of the mammary glands and associated lymph nodes persists for many years.

Bovine brucellosis

- Brucellae may be excreted intermittently in milk for a number of years.
- In bulls, the structures targeted include seminal vesicles, ampullae, testicles and epididymides.
- In tropical countries, hygromas involving the limb joints are often observed when the disease is endemic in a herd.

Bovine brucellosis

- In affected herds, brucellosis can result in decreased fertility, reduced milk production, abortions in susceptible replacement animals and testicular degeneration in bulls.
- Abortion is a consequence of placentitis involving both cotyledons and intercotyledonary tissues.
- In the bull, necrotizing orchitis occasionally results in localized fibrotic lesions.

Diagnosis of Bovine brucellosis

- Clinical signs are not specific although abortions in first-calf heifers and replacement animals may suggest the presence of the disease.
- Clusters of MZN-positive coccobacilli may be evident in smears of cotyledons, and MZN-positive organisms may also be detected in foetal abomasal contents and uterine discharges.

Cont.

- Isolation and identification of *B. abortus* is confirmatory.
- Identification criteria for isolates:

– Colonial appearance

– MZN-positive organisms

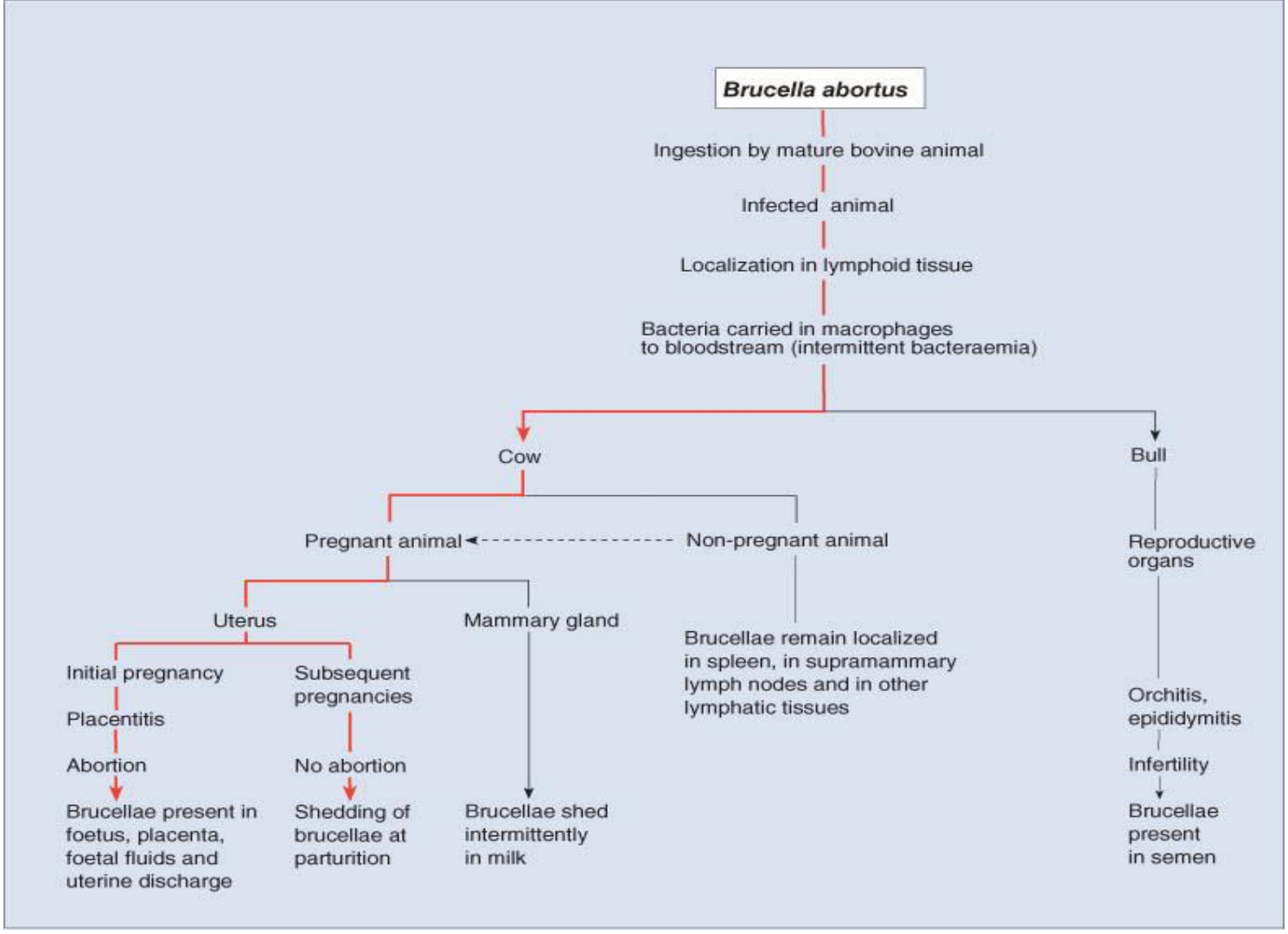
– Bacterial cell agglutination with a high-titer antiserum

- Biotyping using tests and other features indicated
- Rapid urease activity

Cont.

- A range of serological tests, varying in sensitivity and specificity, is available for the identification of infected animals.
- Brucellin, an extract of *B. abortus*, has been used for intradermal testing (Worthington *et al*., 1993).
- Molecular methods, including PCR-based techniques for the detection of brucellae in tissues and fluids, have been developed.

The progression of infection with *Brucella abortus* in mature susceptible cattle


Brucella abortus
Ingestion by mature bovine animal
Infected animal
Localization in lymphoid tissue
Bacteria carried in macrophages to bloodstream (intermittent bacteraemia)
Cow
Bull
Pregnant animal
Non-pregnant animal
Reproductive organs
Uterus
Mammary gland
Brucellae remain localized in spleen, in supramammary lymph nodes and in other lymphatic tissues
Initial pregnancy
Subsequent pregnancies
Placentitis
Orchitis, epididymitis
Abortion
No abortion
Infertility
Brucellae present in foetus, placenta, foetal fluids and uterine discharge
Shedding of brucellae at parturition
Brucellae shed intermittently in milk
Brucellae present in semen

Caprine and ovine brucellosis

- Caprine and ovine brucellosis, caused by *B. melitensis*, is most commonly encountered in countries around the Mediterranean littoral and in the Middle East, central Asia and parts of South America.
- Goats, in which the disease is more severe and protracted, tend to be more susceptible to infection than sheep.

Caprine and ovine brucellosis

- The clinical disease resembles brucellosis in cattle in many respects.
- Clinical features include high abortion rates in susceptible populations, orchitis in male animals, arthritis and hygromas.
- Infection resulting in abortion may not induce a protective immunity.

Diagnosis of Caprine and ovine brucellosis

- Diagnosis is based on clinical signs,
- direct examination of MZN-stained smears of fluids or tissues, isolation and identification of *B. melitensis*
- serological testing.
- Intradermal Brucellin tests are used for surveillance of unvaccinated flocks and herds

Diagnosis

- The Rose-Bengal agglutination test and the complement fixation test are the most widely used methods for detecting infection with *B. melitensis* and are approved for the purposes of international trade.
- Indirect enzyme-linked immunosorbent assays have been developed and are also approved tests for the purposes of international trade

Ovine epididymitis caused by *B. ovis*

- *Brucella ovis* produces an infection in sheep which is characterized by epididymitis in rams and placentitis in Both ram-to-ram and ram-to-ewe venereal transmission occurs.
- Few of the ewes served by an infected ram develop disease.

Ovine epididymitis caused by *B. ovis*

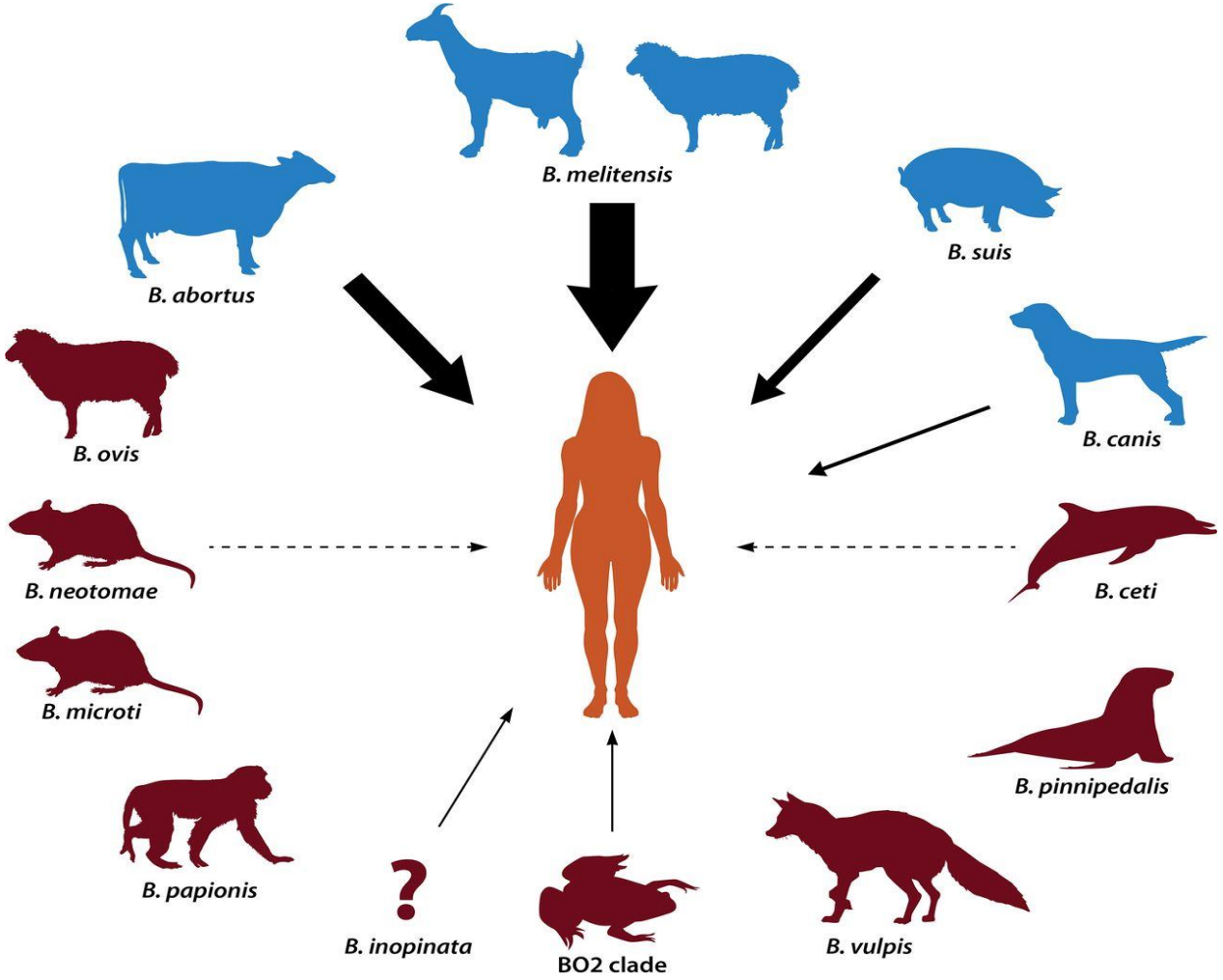
- There is a relatively long latent period in rams following infection.
- *Brucella ovis* may be present in semen about 5 weeks after infection and epididymal lesions can be detected by palpation at about 9 weeks ewes.

Ovine epididymitis caused by *B. ovis*

- Chronically affected rams often have unilateral or bilateral testicular atrophy with swelling and hardening of the epididymis.
- The most efficient and widely used serological tests for *B. ovis* are the agar gel immunodiffusion test, the complement fixation test and the indirect ELISA.
- An immunoblotting technique can also be used as a confirmatory diagnostic test

Brucellosis in humans

- Humans are susceptible to infection with *B. abortus, B. suis, B. melitensis* and, rarely, with *B. canis* and the brucellae of sea mammals.
- Transmission to humans occurs through contact with secretions or excretions of infected animals.
- Routes of entry include skin abrasions, inhalation and ingestion.


B. melitensis
B. abortus
B. suis
B. ovis
B. canis
B. neotomae
B. ceti
B. microti
B. pinnipedalis
B. papionis
?
B. inopinata
BO2 clade
B. vulpis

Brucellosis in humans

- Raw milk and dairy produce made with unpasteurized milk are important sources of infection.
- Laboratory accidents account for some human infections.

Pathogenesis and pathogenicity

- Brucellosis in humans, known as undulant fever, presents as fluctuating pyrexia, malaise, fatigue and muscle and joint pains. Abortion is not a feature of human infection.
- Osteomyelitis is the most common complication.
- Severe infections occur with *B. melitensis* (Malta fever) and *B. suis* biovars 1 and 2.

Pathogenesis and pathogenicity

- Human infections due to *B. abortus* are moderately severe whereas those caused by *B. canis* are usually mild.
- Antimicrobial therapy should be administered early in an infection.
- Humans can develop a severe hypersensitivity reaction following infection or after accidental inoculation with attenuated vaccinal strains.

Question ?