Brucella

By

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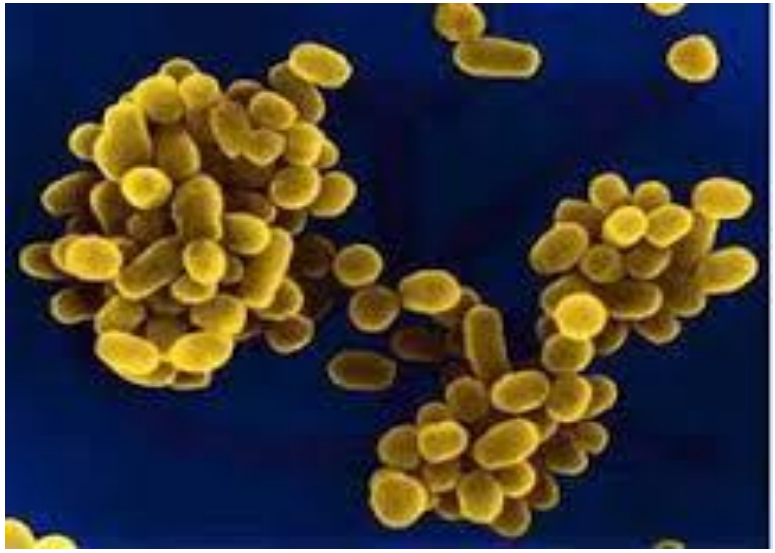
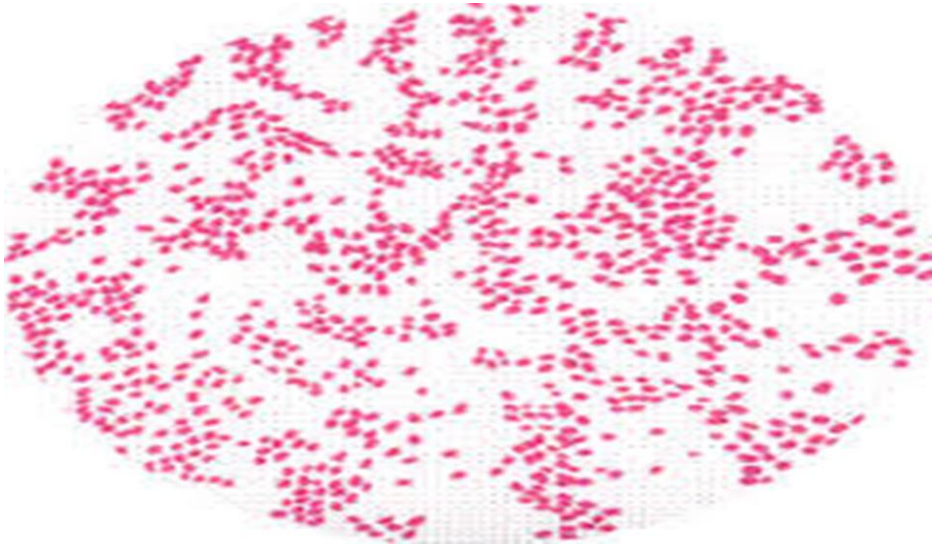
Brucella

Brucella species are small (0.6 x 0.6 to 1.5 pm), non-motile, coccobacillary, Gram-negative bacteria. As they are not decolourized by 0.5% acetic acid in the modified Ziehl-Neelsen (MZN) staining technique, they are classed as MZN-positive. In MZN-stained smears of body fluids or tissues, they characteristically appear as clusters of red coccobacilli.





Key points

Small Gram-negative coccobacilli

Stain red using the modified Ziehl-Neelsen method

Aerobic and capnophilic

Non-motile, catalase-positive

Most isolates are oxidase-positive

Urease-positive

Intracellular pathogens

Target reproductive organs of certain species

Some species cause undulant fever in humans

Usual habitat

As a general rule, *brucellae* 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. Organisms, shed by infected animals, can remain viable in a moist environment for many months.

Types: ***B. abortus, B. melitensis B. ovis , B. canis, B. suis and B. neotomae***

Differentiation of *Brucella* species

Brucella species are differentiated by
1- Colonial appearance,

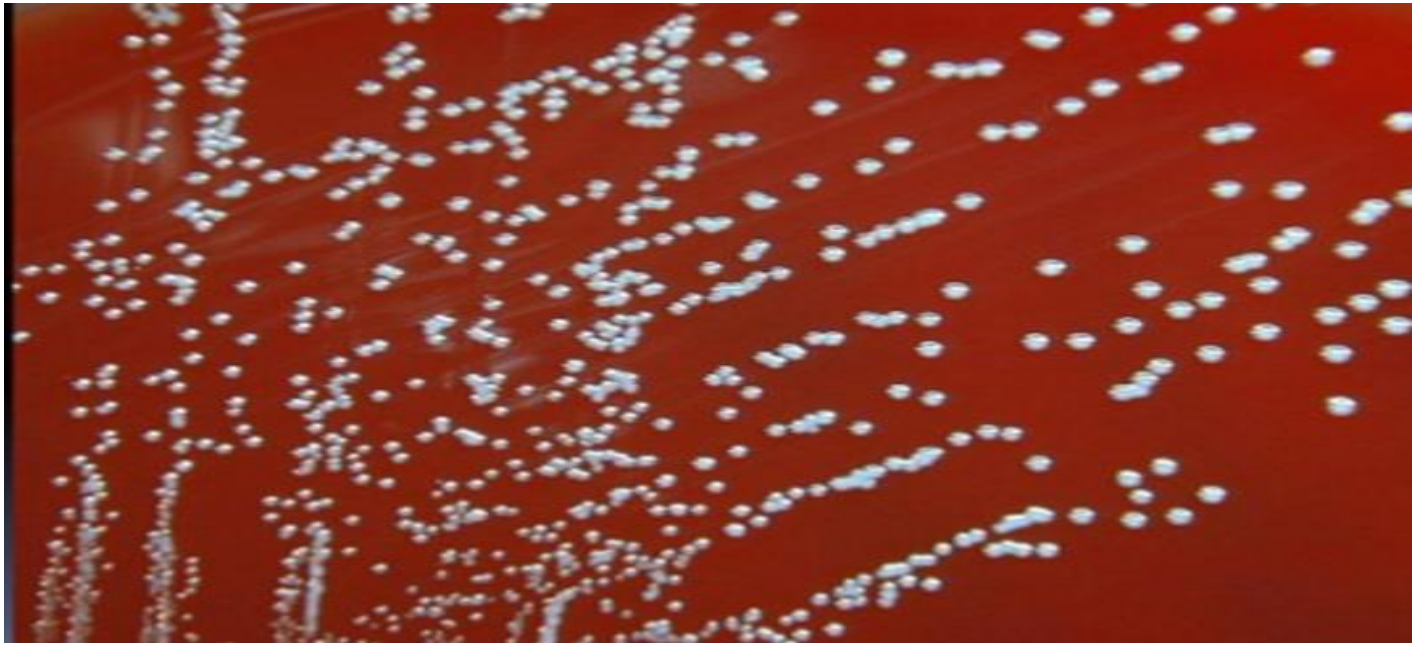
2- Biochemical tests,

3- Specific cultural requirements and

4- Growth inhibition by dyes.
In addition,

5- Agglutination with monospecific sera and

6- Susceptibility to bacteriophages are employed for definitive identification.



On primary isolation, colonies of ***B. abortus, B. melitensis*** and ***B. suis*** occur in smooth forms, and are small, glistening, bluish and translucent after incubation for 3 to ***5*** days. Colonies become opaque with age. In contrast, primary isolates of ***B. ovis*** and ***B. canis*** always occur in rough forms. These rough colonies are dull, yellowish, opaque and friable. Brucellae are non-haemolytic on blood agar.

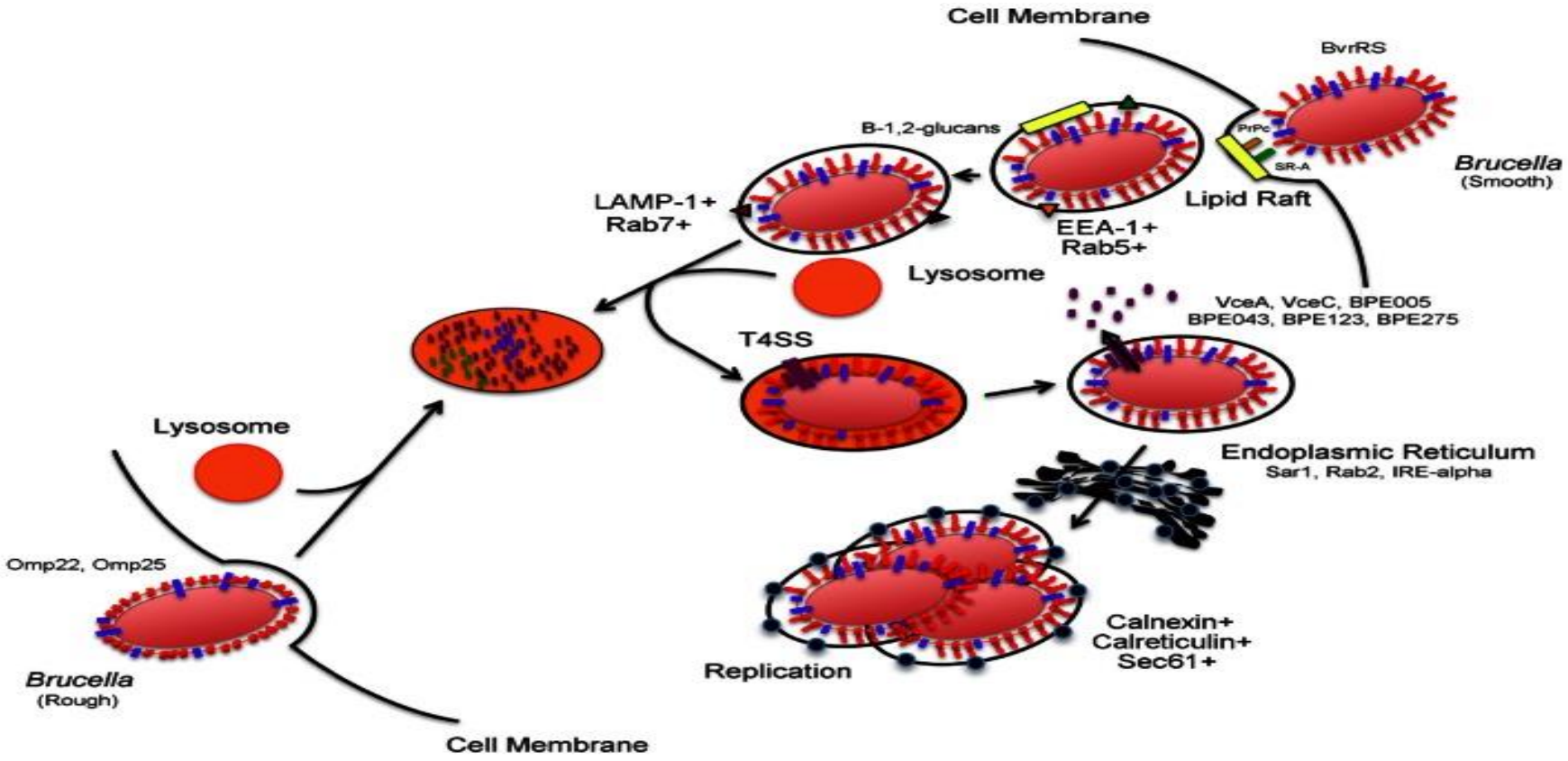
Slide agglutination tests with monospecific antisera are used to detect the presence of important surface antigens, ***abortus*** antigen A and ***melitensis*** antigen M. The R antigen, a feature of the rough brucellae ***B. ovis*** and *B. canis*, can be detected by anti-R serum.

Isolates of *B. abortus* are lysed by a specific bacteriophage (Tbilisi phage) at routine test dilution.

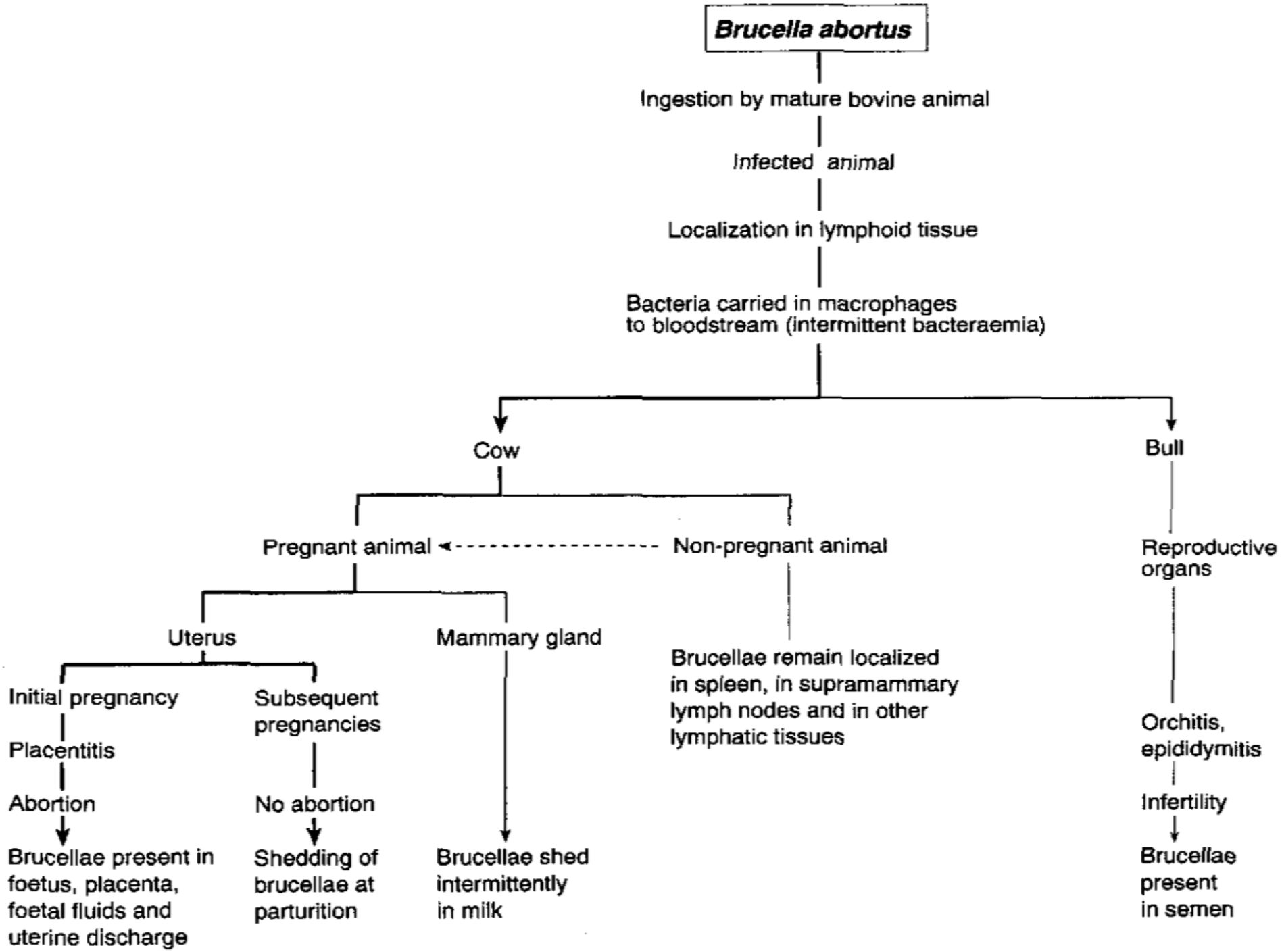
If other tests give equivocal results, oxidative metabolic rates on selective substrates can be conducted in reference laboratories.

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 (Price et al., significance of infection. 1990).



Brucellae, which lack the major outer-membrane lipopolysaccharide, produce rough colonies and are less virulent than those derived from smooth colonies. Although smooth and rough organisms can enter host cells,. Rough forms are usually eliminated unlike smooth forms which may persist and multiply. Virulent brucellae, when engulfed by phagocytes on mucous membranes, are transported to regional lymph nodes. Brucellae persist within macrophages but not within neutrophils. Inhibition of phagocyte-lysosome function is a major mechanism for intracellular survival and an important determinant of bacterial virulence. However, many of the mechanisms used by brucellae to survive within macrophages are not fully elucidated. Various stress proteins are thought to allow the organisms to adapt to harsh conditions encountered within macrophages. In addition, superoxide dismutase and catalase production may play a role in resistance to oxidative killing. 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. This growth factor is also found in other organs such as the mammary gland and epididymis, which are targets for brucellae. In chronic brucellosis, organisms may localize in joints or intervertebral discs.


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
Abortion
No abortion
Orchitis, epididymitis
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

Diagnostic procedures

The diagnosis of brucellosis depends on

1- Serological testing

2- The isolation and

3- Identification of the infecting Brucella species.

Care should be taken during collection and transportation of specimens, which should be processed in a biohazard cabinet.

Specimens for laboratory examination should relate to the specific clinical condition encountered.

MZN-stained smears from specimens, particularly cotyledons, foetalbomasal contents and uterine discharges, often reveal characteristic MZN-positive Coccobacilli

The polymerase chain reaction can be used to detect brucellae in tissues (Fekete ***el al.,*** 1992).

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 IgG1 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

A nutritious medium such as Columbia agar, supplemented with 5% serum and appropriate antimicrobial agents, is used for isolation, Plates are incubated at37°C in 5 to 10% C02 for up to 5 days. Although CO, is a specific requirement for individual species, the majority of brucellae are capnophilic.

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 enterocoliticu***

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

Brucella species	Usual host/clinical significance	Species occasionally infected/ clinical significance
B. abortus	Cattle/abortion, orchitis	Sheep, goats, pigs/sporadic abortion Horses/bursitis Humans/intermittent fever, systemic disease
B. melitensis	Goats, sheep/ abortion, orchitis, arthritis	Cattle/sporadic abortion, brucellae in milk Humans/Malta fever, severe systemic disease
B. suis	Pigs/abortion, orchitis, arthritis, spondylitis, infertility	Humans/intermittent fever, systemic disease
B. ovis	Sheep/epididymitis in rams, sporadic abortion in ewes	
B. canis	Dogs/abortion, epididymitis, disco-spondylitis, sterility in male dogs	Humans/mild systemic disease
B. neotomae	Desert wood rat/not isolated from domestic animals	

Bovine brucellosis

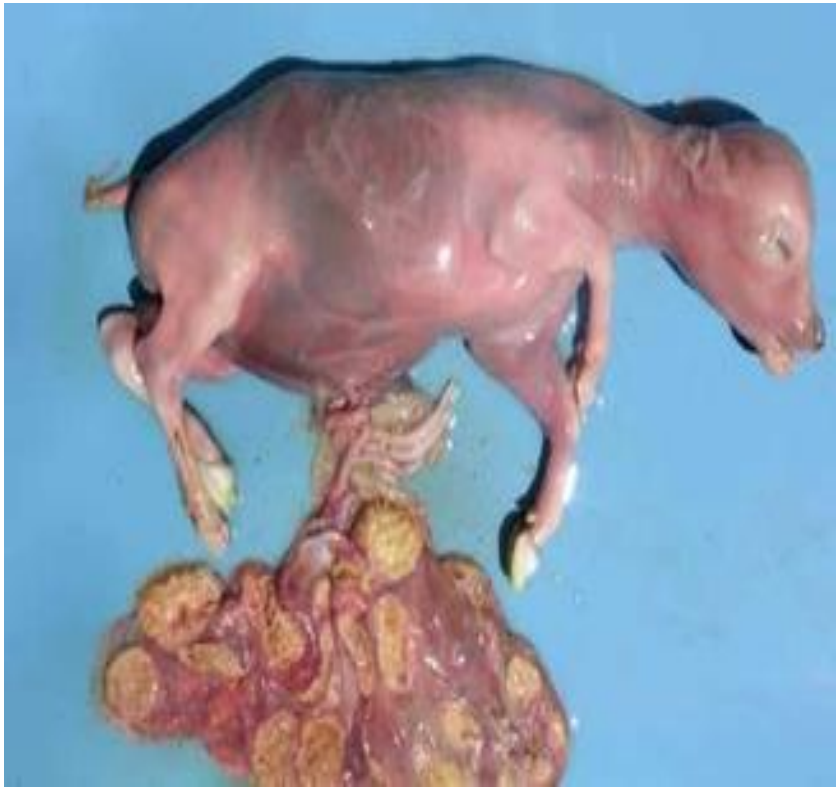
Bovine brucellosis, caused by *B.* ***abortus*** and formerly worldwide in distribution, has been eradicated or reduced to a low prevalence in many countries through national eradication programmes. Although acquired most often by ingestion, infection can occasionally follow venereal contact, penetration through skin abrasions, inhalation or transplacental transmission. Abortion storms may be encountered in herds with a high percentage of susceptible pregnant cows.

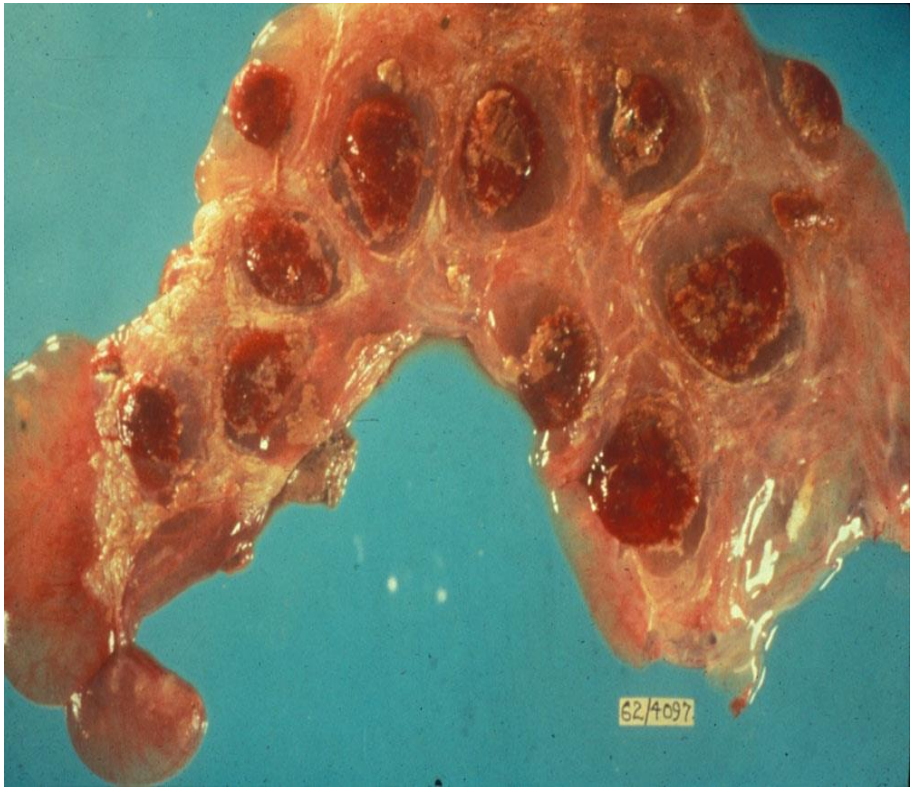
Abortion usually occurs after the fifth month of gestation and subsequent pregnancies are usually carried to term. Large numbers of brucellae are excreted in foetal fluids for about 2 to 4 weeks following an abortion and at subsequent parturitions, although infected calves appear normal.

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.

In affected herds, brucellosis can result in decreased fertility, reduced milk production, abortions in susceptible replacement animals and testicular degeneration in bulls.

Brucellae may be excreted **intermittently** in milk for **a number of years.**

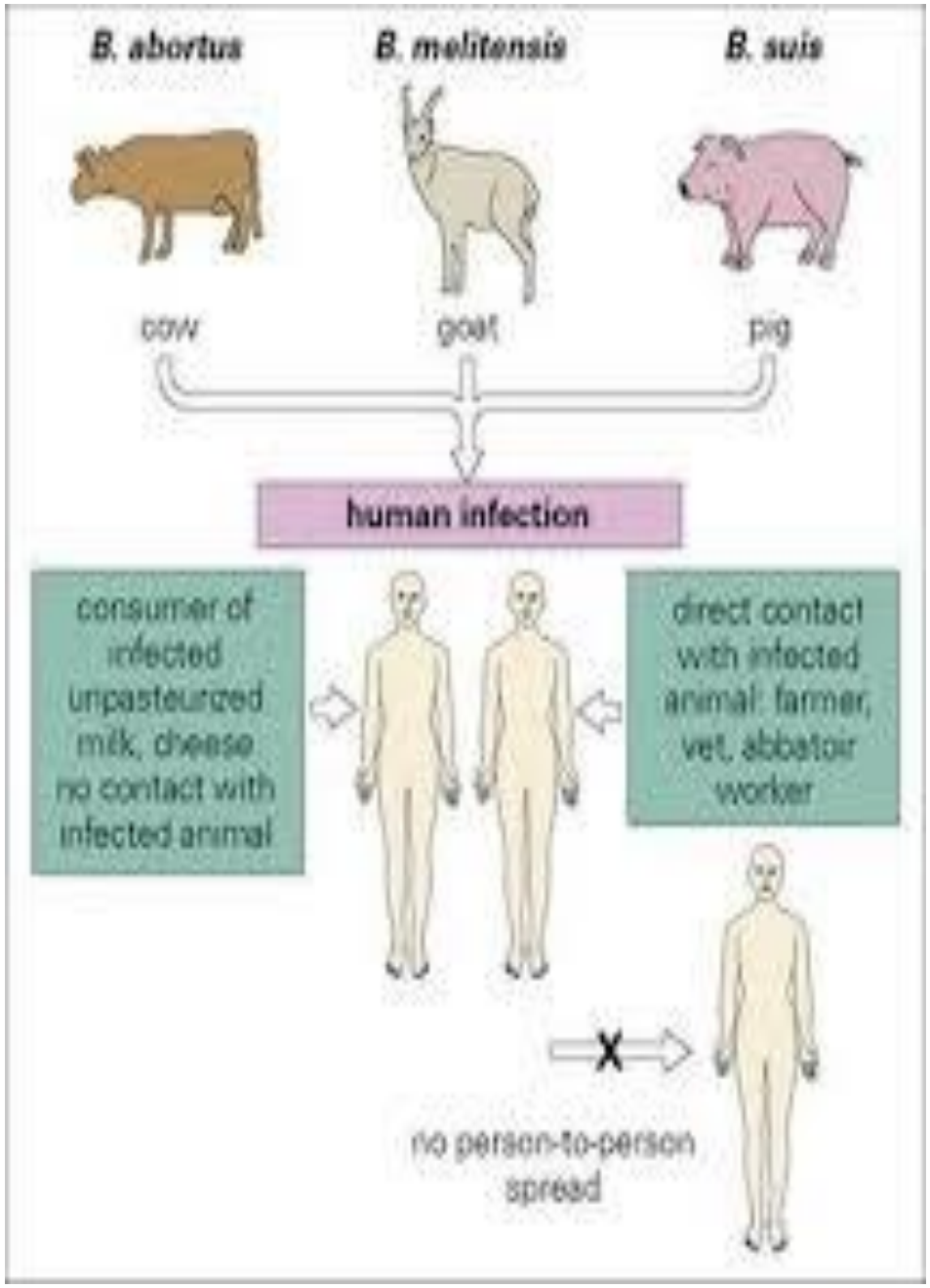


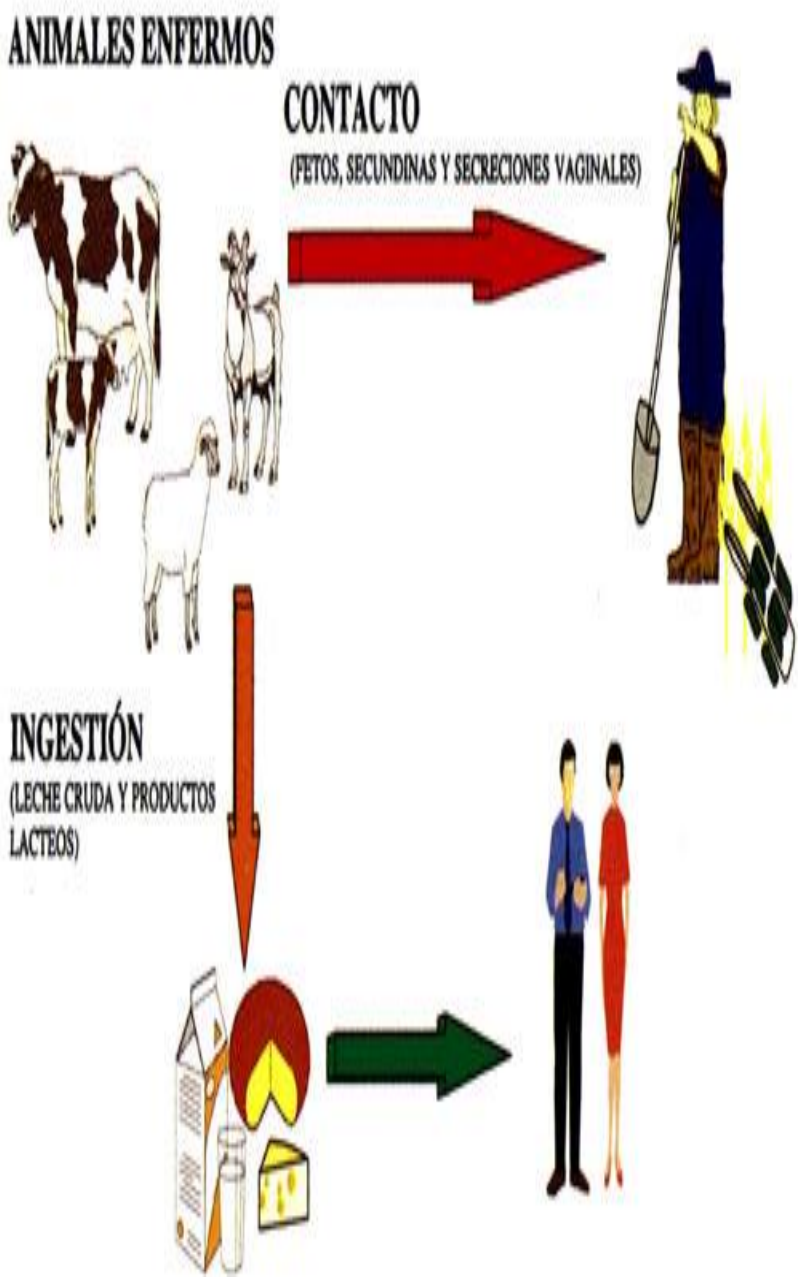


Abortion is a consequence of placentitis involving both cotyledons and intercotyledonary tissues.

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.

In the bull, necrotizing orchitis occasionally results in localized fibrotic lesions.


B. abortus
B. melitensis
B. suis
cow
goat
pig
human infection
consumer of infected unpasteurized milk, cheese no contact with infected animal
direct contact with infected animal: farmer, vet, abbatoir worker
X
no person-to-person spread


ANIMALES ENFERMOS
CONTACTO
(FETOS, SECUNDINAS Y SECRECIONES VAGINALES)
INGESTIÓN
(LECHE CRUDA Y PRODUCTOS LACTEOS)

Diagnosis

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.

Isolation and identification of *B. abortus* is confirmatory.

Identification criteria for isolates:

1-Colonial appearance

2- MZN-positive organisms

3-Bacterial cell agglutination with a high-titred antiserum

4-Rapid urease activity

5-Biotyping using tests and other features indicated in the Table

Brucella species	Number of biotypes	Requirement for CO_2	Production of H_2S	Urease activity	Growth in media containing	
					Thionin (20 μg/ml)	Basic fuchsin (20 μg/ml)
B. abortus	7	v	v	+	v	v
B. melitensis	3	-	-	v	+	+
B. suis	5	-	v	+	+	v
B. ovis	1	+	-	-	+	-
B. canis	1	-	-	+	+	-

v variable reactions related to different biotypes

6- A range of serological tests, varying in sensitivity and specificity, is available for the identification of infected animals (Table 28.3).

7- Brucellin, an extract of ***B. abortus,*** has been used for intradermal testing (Worthington ***et al.,*** 1993).

8- Molecular methods, such as PCR-based techniques, for the detection of brucellae in tissues and fluids

Treatment and control

1- Treatment of cattle with brucellosis is not practical.

2- National eradication schemes are based on the detection and slaughter of infected cattle.

3- Vaccination of young heifers, a strategic measure during the early years of eradication schemes, is discontinued when the prevalence of brucellosis reaches low levels.

Immunity in brucellosis is predominantly cell mediated.

Three types of vaccines are used in cattle:

1-Attenuated strain 19 (S19) vaccine, **2-** adjuvant 45120 vaccine and **3-** the more recent RB51 vaccine:

1- S19 vaccine is administered to female calves up to 5 months of age. Vaccination of mature animals leads to persistent antibody titers.

2-45120 bacterin, although less effective, has been used in some national eradication schemes. Even when administered to adult animals, this vaccine does not induce persistent antibody titers.

3- RB51 strain is a stable, rough mutant which induces good protection against abortion and does not result in serological responses detectable in tests used

Caprine and ovine brucellosis

Caprine and ovine *brucellosis* caused by ***B.*** melitensis, are 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. 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

Diagnosis is based on clinical signs, direct examination of MZN-stained smears of fluids or tissues, isolation and identification of B. melitensis and serological testing. Intradermal brucellin tests are used for surveillance of unvaccinated flocks and herds. In countries where the disease is exotic, a test and slaughter policy is usually implemented. Test and slaughter policies can also reduce the prevalence of disease in endemic areas.

The Rose- Bengal agglutination test and the complement fixation test are the most widely used methods for detecting infection with *B. melitensis*.

Ovine epididymitis caused by *B. ovis*

Brucella ovis produces an infection in sheep which is chacterized by epididymitis in rams and placentitis in ewes. The consequences of infection include reduced fertility in rams, sporadic abortion in ewes and increased perinatal mortality. Both ram-to-ram and ram-to-ewe venereal transmission occurs. Few of the ewes served by an infected ram develop disease. 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. In countries where the disease is endemic, premating checks on rams include serological testing and scrota1 palpation. Chronically-affected rams often have unilateral or bilateral testicular atrophy with swelling and hardening of the epididymis.

Brucellosis in humans

Humans are susceptible to infection with B. abortus, B. suis, B. melitensis and, rarely, with B. canis. Transmission to humans occurs through contact with secretions or excretions of infected animals. Routes of entry include skin abrasions, inhalation and ingestion. Raw milk and dairy produce made with unpasteurized milk are important sources of infection. Laboratory accidents account for some human infections. 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 biotypes 1 and 2. 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.

Species	biotype	Host(s)	Diseases	Geographical distribution
B. abortus	1 2 3 4 5 6	CATTLE' Sheep, goats and pigs Horses Humans	Abortion and orchids Sporadic abortion Associated with baursitis (pollevil and fislulous withers) Undulant fever	Biolypes: 1: Worldwide (common) 2: Worldwide (not common) 3: India, Egypt, East Africa 5: Britain and Germany Other biotypes fare infrequently isolated
B. melitensis	1 2 3	Cattle Humans	Abortion Occasional abortion and excretion in milk Malta fever	Many sheep- and goat-raising regions except New Zealand^ Australia and North America
B suis	1 2 3 4 5	PIGS Humans	Abortion, orchitis, arthritis, spondylitis and herd inlertility Undulant fever	Biotypes: 1: Worldwide 2: Western and Central Europe 3: USA, Argentina and Singapore 4: Arctic Circle (Canada, Alaska and Siberia) in reindeer and caribou
B. ovis		SHEEP	Epididymitis in rams and sporadic abortion in ewes	New Zealand, Australia and some other sheep-raising countries: USA, Romania, Czechoslovakia, South Africa, South America
B.canis		DOGS Human	Abortion, epididymitis, disco-spondylitis and permanent infertility in males Undulant fever	North America and parts of Europe Becoming worldwide but not common
B. neotomae		Desert wood rat (Neotoma lepida)	Non-pathogenic for the wood rat and has not been recovered from any other animal species	USA (Utah)