



**Lecture title: Brucella spp.**

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**Summary: *Brucella spp.***

Key points

1. Classification (According to Bergey's Manual)
2. General characteristics
3. Usual habitat
4. Differentiation of Brucella species
5. Pathogenesis and pathogenicity
6. Diagnostic procedures
7. Treatment and control
8. Brucellosis in humans

Key Points

1. Small Gram-negative coccobacilli
2. Stain red using the modified Ziehl-Neelsen (MZN) method
3. Aerobic and capnophilic
4. Non-motile, catalase-positive
5. Most isolates are oxidase-positive
6. Intracellular pathogens
7. Target reproductive organs.
8. Some species cause undulant fever in humans

1. Classification (According to Bergey's Manual)



Family: Brucellaceae

Genus: Brucella

Brucella abortus

B. melitensis

B. suis

B. ovis

B. canis

B. neotomae

B. ceti

## 2. General characteristics

1. Brucella spp. are small ( $0.6 \times 0.6$  to  $1.5 \mu\text{m}$ ), non-motile,

coccobacillary, Gram-negative bacteria.

2. They stained red by modified Ziehl-Neelsen (MZN). In MZN-stained smears of body fluids or tissues, they characteristically appear as clusters of red coccobacilli.

3. Brucella has six main species. However, new species, including brucellae of sea mammals and voles, have been characterized in recent years (Table 1).

4. Brucella melitensis, B. abortus and B. suis are subdivided into biovars based on cultural and serological properties.

5. Brucella species are aerobic, capnophilic (require 5 to 10%

CO<sub>2</sub>

for primary isolation) and catalase-positive. All Brucella



spp. are urease-positive except *B. ovis*.

6. Media enriched with blood or serum are required for culturing *B. abortus* biotype 2 and *B. ovis*.

**Table 1** *Brucella* species, their host range and the clinical significance of infection

<i>Brucella</i> species	Usual host / Clinical significance	Species occasionally infected / Clinical significance
<i>B. abortus</i>	Cattle / Abortion, orchitis	Sheep, goats, pigs / Sporadic abortion Horses / Bursitis Humans / Intermittent fever, systemic disease
<i>B. melitensis</i>	Goats, sheep / Abortion, orchitis, arthritis	Cattle / Sporadic abortion, brucellae in milk Humans / Malta fever, severe systemic disease
<i>B. suis</i>	Pigs / Abortion, orchitis, arthritis, spondylitis, infertility	Humans / Intermittent fever, systemic disease
<i>B. ovis</i>	Sheep / Epididymitis in rams, sporadic abortion in ewes	—

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**Table 1** *Brucella* species, their host range and the clinical significance of infection

<i>Brucella</i> species	Usual host / Clinical significance	Species occasionally infected / Clinical significance
<i>B. canis</i>	Dogs / Abortion, epididymitis, discospondylitis, sterility in male dogs	Humans / Mild systemic disease
<i>B. neotomae</i>	Desert wood rat / Not isolated from domestic animals	—
<i>B. ceti</i>	Cetaceans	Dolphins / May cause abortion, neurological disease has been described Humans / Little evidence of disease

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### 3. Usual Habitat

1. Brucellae have a preference for both female and male reproductive organs in sexually mature animals and each *Brucella* species tends to infect a particular animal species.

2. Infected animals serve as reservoirs of infection.

### 4. Differentiation of *Brucella* species

*Brucella* species are differentiated by colonial appearance, biochemical tests, specific cultural requirements and growth inhibition by dyes.



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- In addition, agglutination with monospecific sera, susceptibility to bacteriophages and molecular methods are employed for definitive identification.

Slide agglutination tests with monospecific antisera are used to detect the presence of important surface antigens, abortus antigen A and melitensis antigen M.

#### 5. Pathogenesis and pathogenicity

1. Brucellae persist within macrophages but not within neutrophils.
2. Inhibition of phagosome–lysosome function is a major mechanism for intracellular survival and an important determinant of bacterial virulence.
3. In the next phase of infection, virulent brucellae are transported to regional lymph nodes. Intermittent bacteraemia results in spread and localization in the reproductive organs and associated glands in sexually mature animals.
4. 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.
6. Diagnostic procedures
  1. Specimens for laboratory examination should relate to the specific clinical condition encountered.



2. MZN-stained smears from specimens, particularly cotyledons, fetal abomasal contents and uterine discharges, often reveal characteristic MZN-positive coccobacilli. In specimens containing cells, the organisms may appear in clusters.
3. Columbia agar, supplemented with 5% serum and appropriate antimicrobial agents, is used for isolation. Plates are incubated at 37°C in 5 to 10% CO<sub>2</sub> for up to 5 days.
4. Serological testing is used for international trade and for identifying infected herds or flocks and individual animals in national eradication programs

Test	Comments
<b>Brucella milk ring test (MRT)</b>	Conducted on bulk milk samples for monitoring infections in dairy herds.
<b>Rose-Bengal plate test (RBPT)</b>	Useful screening test. Antigen suspension is adjusted to pH 3.6, allowing agglutination by IgG <sub>1</sub> antibodies. Qualitative test only, positive results require confirmation by CFT or ELISA
<b>Complement-fixation test (CFT)</b>	Widely accepted confirmatory test for individual animals
<b>Indirect enzyme linked immunosorbent assay (I-ELISA)</b>	Reliable screening and confirmatory test
<b>Competitive ELISA (C-ELISA) (using monoclonal antibodies)</b>	Recently developed test with high specificity; capable of detecting all immunoglobulin classes and can be used to differentiate infected animals from S19-vaccinated cattle
<b>Serum agglutination test (SAT)</b>	A tube agglutination test which lacks specificity and sensitivity; IgG <sub>1</sub> antibodies may not be detected, leading to false-negative results

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