

## Genus Brucella

**Brucellosis** is a contagious, infectious, and communicable disease, primarily affecting cattle, buffaloes, sheep, goats, swine, and other animals, and is caused by bacteria of the genus *Brucella*, characterized by genitourinary tract infection causing endometritis (abortion storms), retention of placenta, arthritis and orchitis in male. In humans, Malta fever is characterized by an acute septicemic phase followed by a chronic stage that may extend over many years and may involve many tissues.

### Importance and economic implications of Brucellosis in India

**Losses in animal production** due to this disease can be of major economic importance and is primarily because of

- decreased milk production by aborting cows**
- infertility increases the period between lactations** (average inter-calving period may be prolonged by several months.)
- **loss of calves** and
- **interference with the breeding program** and is also important in **beef cattle**.

### Zoonotic Importance

#### Responsible for

- Abortion storms in women
- Orchitis in men rendering men sterile

### Distribution:

Brucellosis is wide spread and of major economic importance in most countries of the world, particularly amongst dairy cattle.

### Brief background of brucellosis situation in animals in India

High Brucella seropositivity states include Punjab, **Maharashtra**, Karnataka, Gujarat, Rajasthan, Uttar Pradesh and Andhra in descending order. **Total economic losses** caused due to brucellosis in animals is US \$3.4 billion, of which 95.6% is constituted by Cattle and buffaloes (2021).

#### Seropositivity in Animals

Species	Sero-positivity
Sheep	11.55%
Cattle	8.3%
Goats	5.37%
Pigs	4.3%
Buffalo	3.6%

### Human Brucellosis

Reported from almost all states

Prevalence: 1.8% in non-occupational & **2.2%** in occupational

Farmers, animal handlers, veterinarians, para-veterinarians, abattoir workers at risk. In a study of 90 cases, 12 cases were diagnosed as **neuro-brucellosis**

## Historical Importance



### Dr. David Bruce (1887)

In 1887 Bruce, a British army physician established the etiology of Malta fever or Melitosis. He described the member of the genus *Brucella* from the case of Malta fever on the island of Malta.

*Brucella* – Bruce; *Melitensis* – Malta fever

He isolated a small microorganism (*Brucella*) from the spleen of fatal cases in Malta & transmitted the disease to monkeys experimentally.

- **Bang** (1897) in Denmark discovered *Brucella abortus* from the cases of abortions in cows.
- **Traum** (1914) found *Brucella suis* from the cases of abortions in swine
- **John Buck - *Brucella abortus* S19** was isolated in **1923** from the milk of a Jersey cow.
- **Cotton W.E.** (1933) The S19 vaccine is a live *Brucella abortus* S19 vaccine developed for use in cattle – calfhood vaccination in female calves only.

## Transmission

*Brucella abortus* achieves its greatest concentration in the contents of the pregnant uterus, the fetus, and the fetal membranes these are the major source of infection.

Transmitted through ingestion, penetration of the intact skin and conjunctiva, by coitus, and by contaminated milk.

**Ingestion of pasture** or other feedstuffs contaminated by discharges from infected cows is by far the commonest method (as organisms can survive and infectivity).

During Vaccination with *Brucella abortus* Cotton Strain 19, if exposed to vaccine accidentally.

## Genus *Brucella*

- Small, non-motile, Gram-negative coccobacilli.
- *Brucella* is intracellular bacteria.
- Grow poorly on ordinary media.
- Optimum pH required is 6.6-6.8.
- Requires aerobic conditions, growth is improved by addition of 5-10 % CO<sub>2</sub> (**Capnophilic**).
- Poor CHO fermentation capability.
- Pathogenic to man and animals.

Important species of *Brucella* and their common hosts:

<i>Brucella abortus</i>	cattle
<i>Brucella melitensis</i>	goats
<i>Brucella suis</i>	pigs
<i>Brucella canis</i>	dogs
<i>Brucella ovis</i>	sheep

### ***Brucella abortus*, *Brucella melitensis* & *Brucella suis***

Three closely related species of *Brucella* are usually discussed as a group and the disease, infection which they produce, is known as brucellosis.

#### **Morphology:**

*Brucella* are coccobacilli or short rods,

Measuring 0.5 to 0.7  $\mu$  by 0.6 – 1.5  $\mu$  in size.

Arranged singly or in short chains.

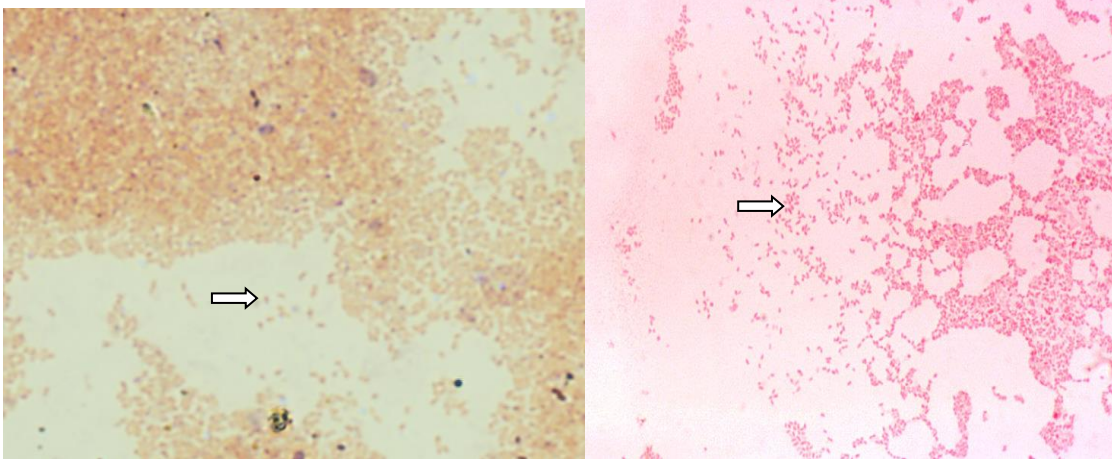
Non-motile,

Non-spore forming,

Non-capsulated.

Gram negative and non-acid fast.

*Brucella* resembles *Hemophilus* and *Bordetella* morphologically.



Microscopic *Brucella abortus*

#### **Growth requirement**

*Brucella* are strict aerobes,

*Brucella abortus* is capnophilic, requiring 5-10% CO<sub>2</sub>, While *Brucella suis* is unaffected.

Optimum temperature 37°C.

Optimum pH 6.6 to 7.4

Can be grown on simple media but liver infusion media is widely used for cultivation of *brucella*.

#### **Cultural characteristics**

**Serum-dextrose agar, serum potato infusion agar, and Trypticase soy agar.**

Addition of polymyxin and cyclohexamide to the above media makes them selective.

#### **Selective media:**

- **Farrell's medium:** Serum dextrose agar containing bacitracin, cycloheximide, nalidixic acid, nystatin, polymyxin B & vancomycin.
- **Brodie and Sinton's medium:** Tryptone soya broth containing amphotericin B & cycloserine + above mentioned selective agents + 5% horse serum.
- **Mair's medium:** Contains gentian violet as selective agent.



**Serum Dextrose agar** : Brucella colonies are small, pale yellow, delicate, translucent and semitransparent. Smooth to rough and mucoid colonies with 1-2 mm in diameter in 24-48 hours.

**Potato agar**: pigmentation is marked; growth appears light yellow, brown or dark brown.

**Mair's medium**, the colonies are blue to violet in colour with an almost black centre.

**Broth**: Organism produces turbidity with fine granular deposits.

**Biochemical Properties:**

- Do not ferment carbohydrates,
- Catalase positive,
- Oxidase positive (except *Brucella ovis* & *Brucella neotomae*)
- Urease positive
- Nitrates are reduced to nitrites,
- Citrate is not utilized
- Indole negative,
- MR & VP negative.

**COUN +ve / CH IMViC - ve.**

**Antigenic properties:**

O Antigen, M & A surface antigen

**Physical Property:**

Can be destroyed by heating at 60°C for 10 mins.

**Pathogenesis:**

- *Brucella abortus* has a **predilection for the pregnant uterus, udder, testicle and accessory male sex glands, lymph nodes, and joint capsules in bursae.**
- Localization in lymph nodes. Spleen. Mammary gland and Iliac lymph nodes.
- **In calves** infection persists in lymph nodes but it is not permanent, as localization does not occur in immature udder and uterus.
- **In adult, non-pregnant cow**, localization occurs in udder and uterus. Infected udder though clinically normal they are important as a source of infection for calves or **humans** drinking the milk.
- When the **invasion of the gravid uterus** leads to severe ulcerative endometritis for the inter-cotyledonary spaces.
- **In male** testis & joint capsules.

## Erythritol

In bovine brucellosis the preferential growth of *Brucella abortus* in certain **foetal materials (placenta, chorion, the fluids)** appeared to be due to the presence in these tissues of erythritol.

Erythritol a **four-carbon polyol / sugar** preferentially utilized by *Brucella*, a **growth stimulant for *Brucella abortus***, Sole carbon source for *Brucella*, is found in the reproductive organs of several affected species, **responsible for the characteristic viscerotropism** of *Brucella* that leads to sterility and abortion. The **presence of erythritol** in the placentas of goats, cows, and pigs has been used to **explain the localization of *Brucella*** to these sites and the subsequent accumulation of large amounts of bacteria, eventually leading to abortion (Collard *et al.*, 2014).

## Intracellular *Brucella*

- *Brucellae* ingested by mononuclear phagocytes survive and replicate initially. **Intracellular survival within macrophages is facilitated by inhibition of phagosome-lysosome fusion by soluble products of *brucellae*, and production of stress-induced proteins.**
- Eventual elimination of virulent *brucellae* depends on activation of macrophages through development of TH-1 type cell-mediated immunity.
- Cytokines contributing to anti-brucella activity of activated macrophages include: TNF- $\alpha$ , TNF $\gamma$ , IL-1, IL-12.

## Clinical Signs :

### Cows:

Abortion in last trimester, Retention of placenta, Acute Septicaemia

### In Bulls

Orchitis and epididymitis – One or both testis may be affected

Painful swelling to twice the normal size.

Testis –liquefaction and fibrosis

Non-suppurative synovitis, fistulus withers, poll evil.

## Human being:

### Symptoms

Malaise, Chilling, fever 7-21 days after infection

Drenching sweats in the late afternoon or evening

Periodic nocturnal fever

Bodyache, headache, occasionally localized infections. Orchitis & abortions.

### Long-term signs and symptoms may include:

Fatigue, Recurrent fevers, Inflammation of the inner lining of the heart chambers (endocarditis)

Joint inflammation (arthritis)

Arthritis of the spinal bones (spondylitis)

Arthritis of joints where the spine and pelvis connect (sacroiliitis)

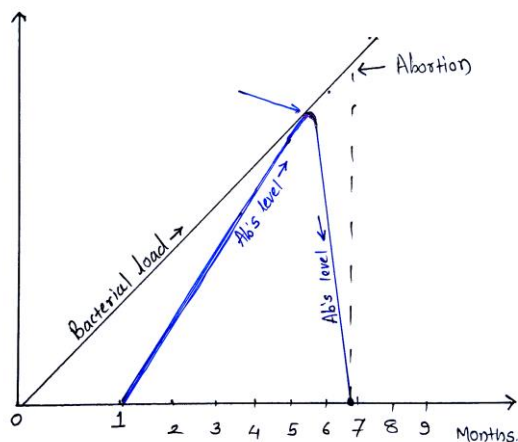


Fig.: Brucellosis: Bacterial Load and Antibody Level

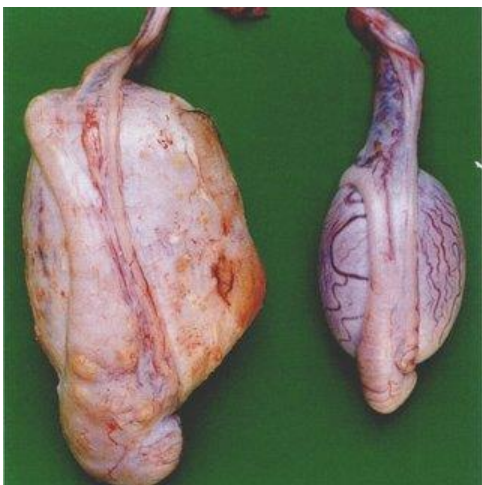


Fig.: Orchitis (Left); Right testis normal



Fig.: Abortion and Placentitis

**Prevention:**

- Use of vaccine :  
*Brucella abortus* Cotton strain-19 calf hood vaccine  
*Brucella abortus* 45/20 vaccine  
 Rev 1 Sheep and goat.

**Control:**

- Identification and **elimination of infected animal**
- Hygienic disposal of an aborted fetus and fetal membranes
- Quarantine
- Use of vaccine:  
 Cotton strain-19 calf hood vaccine  
*Brucella abortus* 45/20 vaccine- Used in adult cattle  
 Rev 1 for Sheep and goat.

## Diagnosis of Brucellosis:

### Material to be collected:

Paired serum, heart blood, and abomasal contents of aborted fetuses, placenta with 2-3 cotyledons, vaginal swabs in PBS, in separate bottles on ice, whole fetuses, if small, on ice. Neat semen in a sterile vial or semen straw on ice. (Serum sample collected immediately after abortion may test false negative, hence paired sera)

### 1. Isolation & identification of Brucella organisms

#### Trypticase Soy Agar/Serum Dextrose Agar

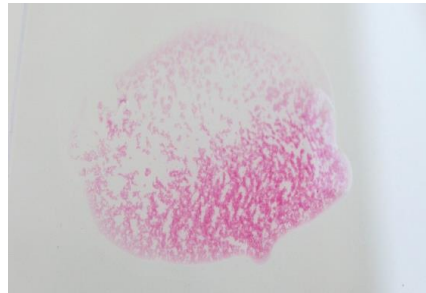
**Cultural characteristics: Serum Dextrose agar:** Brucella colonies are small, pale yellow, delicate, translucent, and semitransparent. Smooth to rough and mucoid colonies with 1-2 mm in diameter in 24-48 hours.

**Microscopic:** Gram negative coccobacilli, non-capsulated, non-spore forming. Non-motile

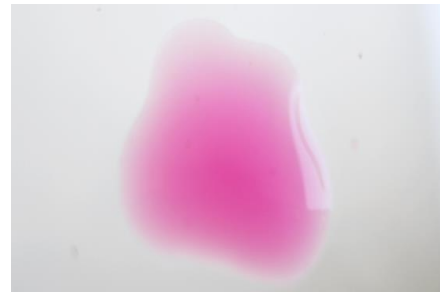
**Biochemicals: COUN Positive; CH(Carbohydrate) IMViC Negative**

### 2. Rose Bengal Plate Test:

The Rose Bengal Plate test (RBPT) is a rapid slide-type agglutination assay performed with a stained *B. abortus* suspension at pH 3.6–3.7 and plain serum. Because of its simplicity, it is often used as a screening test on a herd basis.



Brucella positive RBPT test  
Clump formation



Brucella negative RBPT test  
No clump formation

### 3. Serum Agglutination Test:

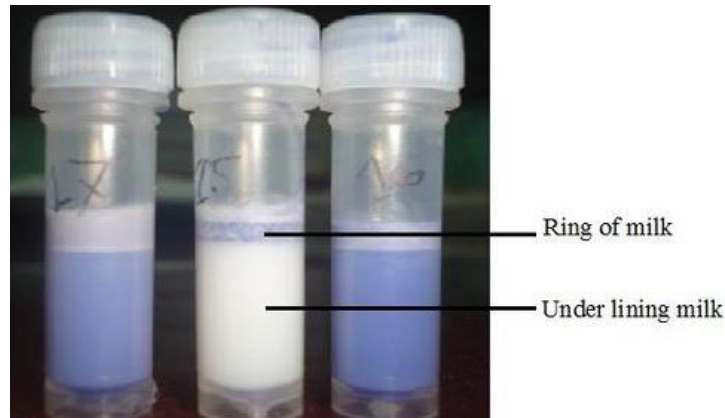
**Serum tube agglutination test (SAT)** is the standardized gold method, it is laborious, time-consuming, and requires a number of reagents. A microagglutination test (MAT) variant of the SAT or enzyme-linked immunosorbent assay (ELISA) is recommended for serological diagnoses. For the simple and rapid diagnosis of brucellosis, requires less test sample, and reagents.

**Microagglutination test (MAT):** A microagglutination test (MAT) variant of the SAT. For MAT, commercial *Brucella* antigen, in 96-well U-shaped microplates. Serial two-fold dilutions of the sera are made in saline from 1:10 and to 1:1280. The MAT is performed by incubating the sera at 37°C for 24 h.

To assess the titers of Brucella: Sheep and Goat 1:20; Cattle above 1:40; Human above 1:80

#### 4. Milk Ring Test:

One drop of (0.03ml) stained brucella antigen standardized against international standard serum is added to 1 ml of whole milk that has been kept in the refrigerator overnight. Read the test after incubation for 1 hour at 37° C. A positive reaction is indicated by a stained cream layer over the white column of milk.



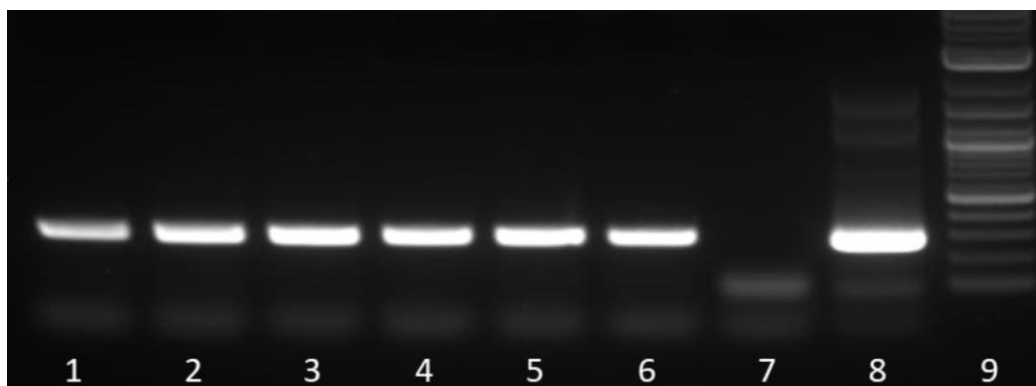
**Milk Ring Test:** Milk in the middle tube indicates a positive result showing the ring of cream is more colored than the underlying milk. Milk in the two corner tubes indicates a negative result showing the ring of cream less colored than the underlining milk. (Sarker *et al.*, 2014)

#### Principle

Based on the principle of agglutination between antibodies contained in milk and colored bacterial antigen of brucella (hematoxylin or eosin stained antigen) to form antigen-antibody complexes that are progressively carried by the fat towards the surface of the milk and form a blue-violet ring.

#### 5. Polymerase Chain reaction

The most sensitive and rapid test in the diagnosis of Brucellosis. Amplification of 16S rRNA sequences of *Brucella abortus* or *omp* gene or *bcs*p gene molecular diagnosis with high precision or sensitivity can be achieved.





## 6. Enzyme-linked immunosorbent assay (ELISA)

**ELISA of IgG and IgM** are performed and interpreted using a commercial kit. An index value is calculated to generate the results for either IgG or IgM as follows; negative, <9; equivocal, 9 to 11; and positive, >11. **Very sensitive test used to differentiate whether the infection is active/latest or chronic.**

**7. Brucella Coombs Gel Test:** A new serological method is identified as Brucella Coombs gel test based on the principle of centrifugation gel system similar to the gel system used in blood group determination. In this system, if Brucella antibodies were present in the serum, antigen and antibody would remain as a pink complex on the gel. Otherwise, the pink Brucella antigens would precipitate at the bottom of the gel card system (Hanci *et al.*, 2017).

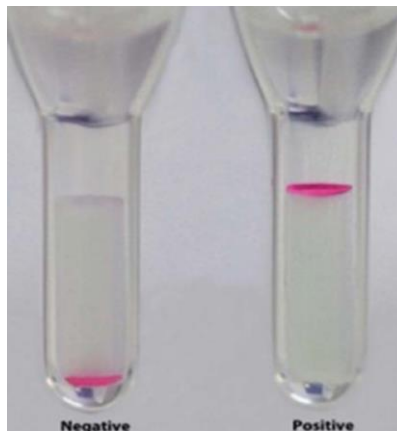


Fig. Negative and positive results in the Brucella Coombs gel test

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## National Brucella Control Programme 2022-23

- **Live B. abortus strain 19 vaccine**
- It is the most effective vaccine produced to date.
- S19's efficacy is estimated to be ~70% (Lubroth *et al.*, 2007).
- The S19 vaccine is normally given to **female calves aged between 4 and 8 months** as a **single subcutaneous dose of  $5-8 \times 10^{10}$  viable organisms**. Because this vaccine develops persistent antibody titers, **brucellae may be excreted into milk and are abortive in pregnant cattle** (Stevens *et al.*, 1994).
- A **significant disadvantage** of S19 is its smooth phenotype making it difficult to distinguish between vaccinated and naturally infected animals using common serological diagnostic tests.
- S19 can cause abortion if used in pregnant cattle, and it is **fully virulent for humans** (Yang *et al.*, 2013).
- **Personal protective equipment (PPE) should be used while vaccinating the female calves.**

## Live *B. abortus* strain 19 vaccine

### Accidental exposure to *Brucella abortus* S19 vaccine

Accidental parental injection of vaccine into human body

Spill and exposure to oral mucosa or eye

Exposure of open wound/Break in skin

Consumption of milk from adult vaccinated animal

### Accidental exposure to *Brucella abortus* S19 vaccines

Administration of two synergistic antibiotics,

doxycycline and rifampicin or doxycycline, and an aminoglycoside, is normally recommended, the treatment should last a period of at least six weeks. The discontinuity of chemotherapy is responsible for debilitating complications and relapses.

### When to see a doctor?

Brucellosis can be hard to identify, especially in the early stages, when it often resembles other conditions, such as the flu.

See your doctor if you develop a rapidly rising fever, muscle aches, or unusual weakness and have any risk factors for the disease, or if you have a persistent fever.

History of exposure.

### Brucella Vaccine S19 If given Accidentally

**Adult Pregnant animal:** the potential to cause abortion in pregnant animals

**In Males:** May be localized in the testis leading to related complications.

### Drawbacks of using *Brucella abortus* S19 Vaccine

Interference with diagnostic tests, Pathogenicity for humans.

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