

BRUCELLOSIS

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Introduction

1. Brucellosis is considered as one of the most **wide spread zoonosis** in the world.
2. The importance of this contagious disease is the **economic impact** on livestock industry.
3. Causes **severe hazard to human health**, through either direct contact with infected animals or the consumption of contaminated milk and dairy products.



Brucellosis: Synonyms

Human Disease

- Malta Fever
- Undulant Fever
- Mediterranean Fever
- Rock Fever of Gibraltar
- Gastric Fever

Animal Disease

- Bang's Disease
- Enzootic Abortion
- Epizootic Abortion
- Slinking of Calves
- Contagious Abortion
- Ram epididymitis



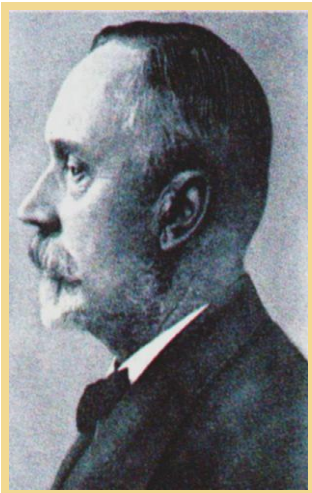
HISTORY



Sir David Bruce (1855-1931)

- British Army physician and microbiologist who discovered *Micrococcus melitensis*

Nomenclature today is credited to **Sir David Bruce**
Brucella abortus and *Brucella melitensis*



Bernhard Bang (1848-1932)

- Danish physician and veterinarian Discovered *Bacterium abortus* could infect cattle, horses, sheep and goats



BRUCELLA ORGANISM

- *B. abortus* (cattle)
- *B. melitensis* (sheep & goats)
- *B. ovis* (sheep)
- *B. suis* (pigs)
- *B. canis* (dogs)
- *B. neotomae* (wood rat)



Brucella species :

S.N.	Species	Biovar	Natural host
1.	<i>Brucella abortus</i>	1-9	Cattle
2.	<i>Brucella melitensis</i>	1-3	Sheep and Goat
3.	<i>Brucella ovis</i>	1	Sheep (Ram)
4.	<i>Brucella suis</i>	1-5	Swine
5.	<i>Brucella canis</i>	1	Dog
6.	<i>Brucella neotomae</i>	1	Wood rat

Organisms

- Brucella are small ($0.4-0.8 \times 0.5-1.5\mu\text{m}$), non-motile, non-capsulate, **gram-negative** coccobacilli.
- The organism is **aerobic to microaerophilic**.
- **Intracellular**
- All strains grow best in a medium enriched with animal **serum** and **glucose**



Biochemistry

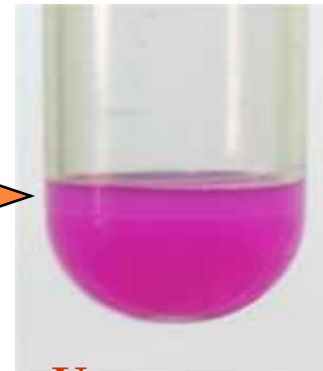
- Oxidase **+ve** →



Oxidase test

- Nonfermentative

- Urease **+ve** \ catalase **+ve** →



Urease test



Catalase test

- **H₂S** produced by *B. abortus* and *B. suis*

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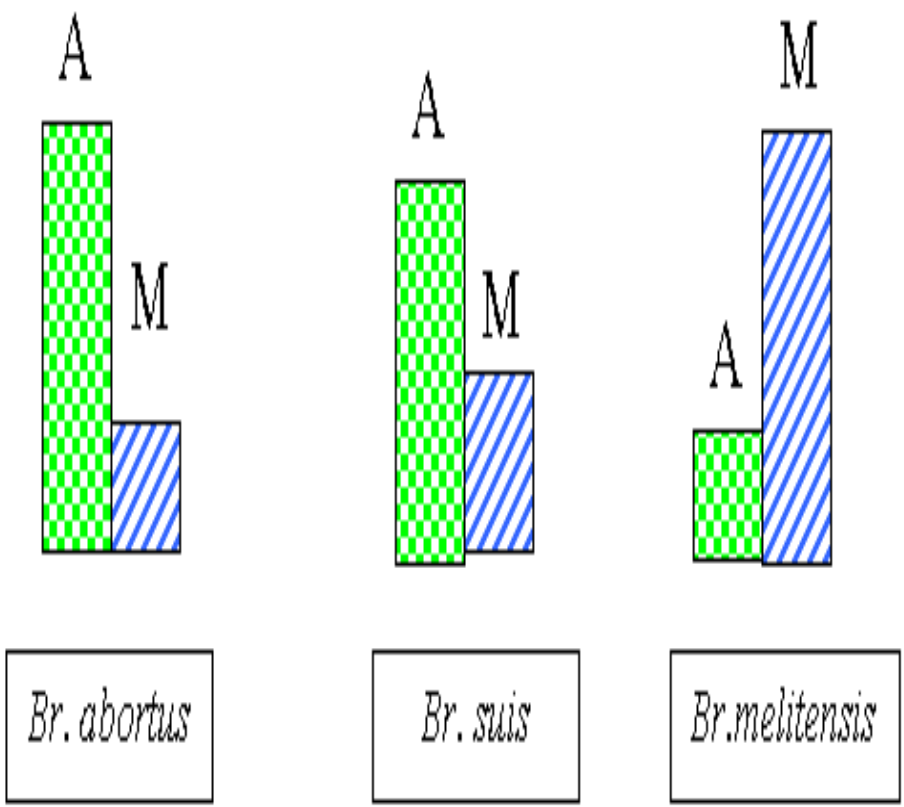
ANTIGENIC STRUCTURE AND CLASSIFICATION

- Two main antigen: **A** and **M**
- The three main Brucella differ from one another in the amount of the two main antigen they have in common :

B.abortus : **A:M=20:1**

B.melitensis: **A:M=1:20**

B.suis: **A:M=2:1**



The difference in ratio between A and M Antigens

The designation of the antigens in cultures composed of **smooth and rough colonies**

NO.	Species	Type of colony	Type of surface antigen		
			A	m	R
1.	<i>Brucella abortus</i>	Smooth	A	m	---
2.	<i>Brucella melitensis</i>	Smooth	a	M	---
3.	<i>Brucella suis</i>	Smooth	A	m	---
4.	<i>Brucella ovis</i>	Rough	---	---	R
5.	<i>Brucella canis</i>	Rough	---	---	R

- The Production of **monospecific antisera** to A and M antigen can be used in the identification of the Brucella species.
- *Br.canis* and *Br.ovis* grow as **rough colonies** that possess **R antigen**.



RESISTANCE

- The organism killed at 60⁰c
- IN milk = killed by the pasteurization
- In foetus = for several month
- Organism susceptible to the sunlight, Acidic pH, disinfectant.
- Can survive in soil for 70 days and for about 35 days in water.




WHO ARE AT RISK?

- ✓ Cattle ranchers/ dairy /pig/sheep and goat farmers
- ✓ Veterinarians
- ✓ Abattoir workers
- ✓ Meat inspectors & Lab workers
- ✓ Consumers of unpasteurized dairy products



TRANSMISSION IN ANIMALS

- Ingestion of infected tissues or body fluids, contaminated feed or water, or after licking an infected placenta, foetus or genitalia of another cow, after it has aborted.
 - Infected bulls may excrete the organism in their semen.
 - Congenital transmission may occur through in utero infection.
 - Contact with infected tissues or body fluids: Mucous membranes, injections.
 - Venereal: Swine, sheep, goats, dogs and other species.
- 

TRANSMISSION TO HUMANS

- **Conjunctiva** or **broken skin** contacting infected tissues
 - Blood, urine, vaginal discharges, aborted fetuses, placentas
- **Ingestion**
 - Raw milk & unpasteurized dairy products
 - Rarely through undercooked meat
- **Inhalation** of infectious aerosols



CULTURAL CHARACTERISTICS

- Brucella colonies are visible after **3-5 days** incubation period at 37°C.
- They are **aerobic** or **microaerophilic**.
- Brucella colonies are 1-2 mm in diameter, round, entire, smooth, glistening, translucent, and a pale honey color when plates are viewed in the daylight.
- All strains grow best in a medium enriched with **animal serum** and **glucose**
- Require **5-10% carbon dioxide** (except *B. melitensis*)



Brucella colonies on BA

Culture media :

There are two major types of media for cultivation of Brucella:

A. Basal Medium

- **commercial media** ,e.g. Brucella medium base, Trypticase soy agar, Columbia agar, Serum- dextrose agar or Glycerol-dextrose agar.
- The addition of 2-5 % **Bovine or Equine serum** is necessary for the growth of strains such as *B. abortus*.

B. Selective Media

- Most widely used medium is **Farrell's medium**, prepared by the addition of six antibiotics:

Polymyxin B sulphate, Bacitracin, Cycloheximide, Nalidixic acid, Nystatin, Vancomycin

Skirrow agar for Brucella and Campylobacter

Why Brucella mainly affects genital and mammary tissues ?

- Erythritol, a polyhydric alcohol which acts as a growth factor for brucellae, is present in high concentrations in the placenta of cattle, sheep, goats and pigs. (Thus the brucella organisms are having affinity for gravid uterus.)
- Also present in mammary gland and epididymis.
- In chronic cases organisms localize in joints or intervertebral disc.



BRUCELLOSIS: ANIMAL DISEASE

- Chronic infection *Brucella*
- Infect organs rich in **erythritol** like udder, uterus, placenta and epididymis
- **Clinical Signs:** abortion and sterility
- **Asymptomatic carrier** :Shed large numbers of organisms in milk, urine, and reproductive tissues



B. abortus infection: in cattle

- **Third trimester** abortions with *B. abortus*
- Retained placenta
 - Once expelled will have a **leathery appearance**
- Birth of dead or weak calves
 - Respiratory distress and lung infections
- Low milk yield Will shed organisms in milk for life
- In male-orchitis & epididymitis



B. melitensis causing: Sheep and Goat

- *B. melitensis* causing **late term abortions**
 - Retained placenta
 - Birth of dead or weak lambs/kids
- Goats - articular and periarticular **hygroma** localizations
- *B. ovis* causing abortions, fertility problems
 - **Orchitis, epididymitis**
 - Abnormal breeding soundness
 - Organisms present in semen



B.suis: Swine

- Prolonged **bacteremia**
- Abortion, **early or late** gestation
- Fertility problems
 - Sows temporary
 - Boars- unilateral or bilateral orchitis
- Lameness, posterior paralysis, spondylitis, metritis, abscesses



B. canis: Dogs

- Susceptible to
 - *B. melitensis*, *B. abortus*, *B. canis* and *B. suis*
- *B. canis* causes abortions
 - **Last trimester** of pregnancy
 - Prolonged vaginal discharge
 - **Bacteremia** for up to 18 months post-exposure
 - Failure to conceive, stillbirths, prostatitis, epididymitis



DIAGNOSIS

- Direct microscopic examination
- Isolation of organism
 - Blood, semen, other tissues
- Serology
- Brucella milk ring test
- Biological test
- Demonstration by fluorescent antibody of organism in clinical specimen
 - Placenta, fetus



Direct microscopic examination:

- Prepare the impression smear (samples like cotyledons, aborted fetal stomach content or spleen, uterine discharge)
- Perform gram's staining staining
- Examine under microscope
- On examination they may be found in neutrophils or macrophages
- **Koster's staining:** To demonstrate brucella in smear from placenta



Isolation

- Most preferred samples: cotyledons, aborted fetal stomach content or spleen, uterine discharge, milk and semen
- Inoculate the sample on dextrose agar or blood agar, with 5-10% CO₂ for 3-6 days.



Brucella colonies on BA

The most common serological test

1. Rose Bengal Test (**RBT**).
2. Milk Ring Test (**MRT**) /Herd test.
3. Standard Tube Agglutination Test (**STAT**).
4. Enzyme Linked Immuno Sorbent Assay (**ELISA**).



Rose Bengal Test (RBT)

A. Principle

The buffered – acid antigen stained with Rose Bengal is used for the early detection of Brucella specific agglutinins.

B. Reagents

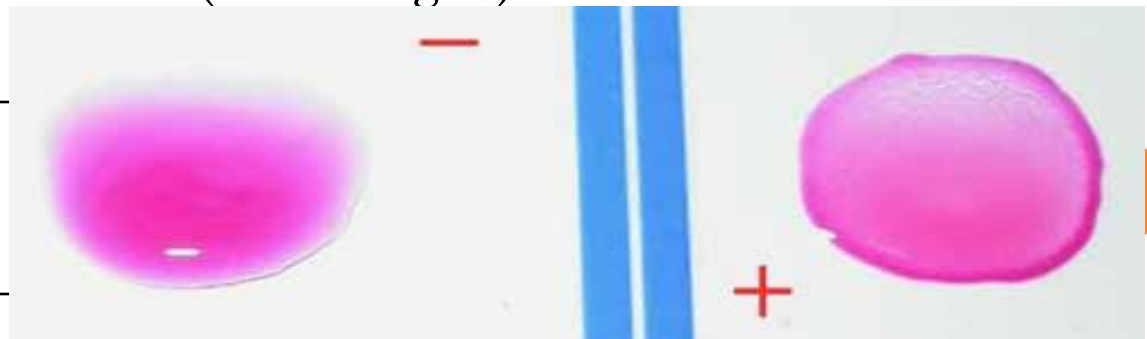
Rose Bengal Antigen

Result:

No agglutination = Negative result.

visible agglutination (even slight) = Positive results

FIG: POSITIVE AND NEGATIVE RESULTS FOR RBT (ROSE BENGAL TEST)



MILK RING TEST (MRT)



The Ring test method is used for the detection of brucella antibody in milk samples. This technique is very easy to perform it especially in **dairy herds**.

A. Principle

It is uses the principle of **agglutination** between antibodies contained in milk and dyed colored bacterial antigen of brucella to form antigen-antibody complexes that are progressively carried by the fat towards the surface of the milk and formed a blue violet ring

B. Reagents

- Inactivated bacterial culture of *Brucella abortus* S 99 stained with hematoxyline.

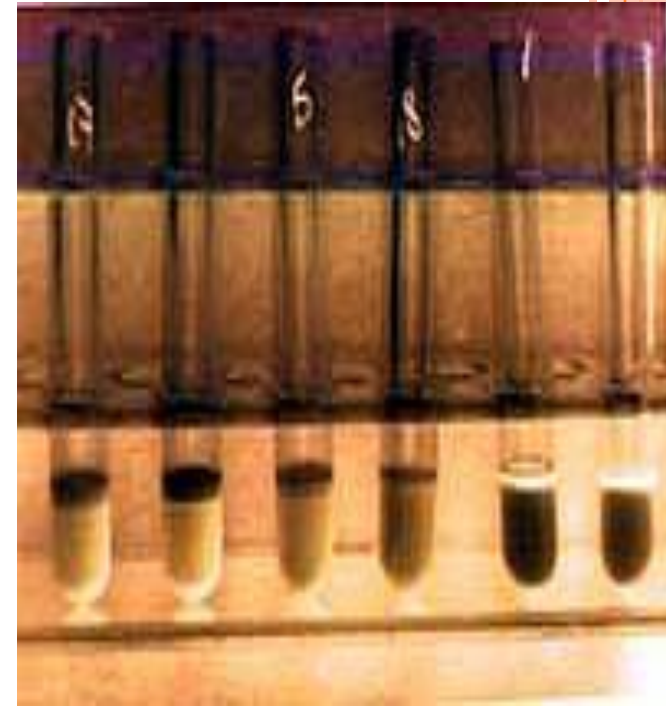
Procedure



1. Carefully mix the tested milk, and put 1 ml in a test tube.
2. Add 50 μ l of the antigen and mix carefully.
3. Place in incubator for 1 hour at 37 $^{\circ}$ C, after that for (18-20 hours) at 4 $^{\circ}$ C, and then read the result.

Results

1. Ring of cream equal or more colored than the underlying milk
= Positive result.
2. Ring of cream less colored than the underlying milk
= Negative result.



STAT: Standard Tube Agglutination test

- Quantitative test
- Titre is calculated on the basis of this test which is used to cull or slaughter the animals
- In this Colourless Brucella antigen is mixed with different dilution of serum, to quantitate the antibodies present in the suspected serum of animals.



DIFFERENT CHARACTERS TO IDENTIFY THE SPECIES



species	CO2 incubation	H2S production	Urea hydrolysis	Growth in Carbal/basic fuschin	Growth in Thionine	Growth in Malachite green
<i>B. abortus</i>	required	2-4 days	Very slow	+	-	+
<i>B. suis</i>	Not required	7 days	rapidly	-	+	-
<i>B. melitensis</i>	Not required	1 day	slowly	+	+	+

- **Dye inhibition test:** The species of brucella have differences in ability to grow in medium containing basic fuschin, thionine and malachite green
- RTD(Routine test dilution): Tbilisi phage

Thank You

