

BRUCELLA

Learning objectives

To know in detail about,

- Diseases produced by the pathogenic brucella in domestic animals
- Morphology, cultural and biochemical characters of *Brucella sp*
- Selective media for Brucella
- Antigens and biotyping of Brucella
- Pathogenesis of brucellosis in domestic animals
- Different diagnostic methods of brucellosis
- ABRT, Coombs test, RBPT, Card test and Whey agglutination test
- General approaches used to control brucellosis

SYSTEMATICS

Domain	Bacteria
Phylum	Proteobacteria
Class	Alpha proteobacteria
<u>Order</u>	Rhizobiales
Family	Brucellaceae
Genus	Brucella

- Brucella are small Gram negative, non-motile, non-spore forming rods.
- They are aerobic and carboxyphilic. Catalase, oxidase and urease positive.
- They are partially acid fast, in that they are not decolourised by 0.5% acetic acid in the Modified Ziehl –Neelsen acid stain.
- All the six recognised species, *B.abortus*, *B.melitensis*, *B.suis*, *B.ovis*, *B.canis* and *B.neotomae* are pathogenic, facultative, intra cellular parasites with a predilection for the reticuloendothelial system and the reproductive tract.

HISTORY AND HABITAT

History

- *B.melitensis* was identified by Bruce in Malta in 1887. *B.abortus* was first recognised by Bang in 1897. *B.suis* was discovered by Traum in 1914.
- Alice Evans (1918) identified the first *Brucella* of human origin in the USA. Buck (1930) developed live attenuated strain 19 vaccine.

Habitat

- *Brucella* species are obligate parasites and each species has a preferred natural host that serves as a reservoir of infection.
- Brucellae have a predilection for ungulate placentas, foetal fluids and testes of bulls, rams, boars and dogs.

MORPHOLOGY AND CULTURAL CHARACTERISTICS

Morphology

- Brucellae are coccobacilli or short rods, arranged singly or in short chains. They are non-motile, non-capsulated, non-sporing and partial acid fast.
- They are stained by Grams, MZN and Koster's stain.
- Koster's stain is mostly useful in demonstrating Brucella in smears from the cotyledons in bovine abortion (Cells of chorion are packed with organism).
- MZN is especially useful to stain smears from foetal membranes, uterine swabs, smear or stomach contents of aborted foetus.

Cultural characteristics

- Brucella is strict aerobe.
- *B.abortus* is carboxyphilic, requiring 5-10% CO₂.
- The optimum temp. is 37°C. The growth very slow on simple media, the plates should be incubated for as long as 30 days.
- Growth is improved by addition of serum or liver extract.
- Growth is also improved by addition of several aminoacids, thiamine, biotin, magnesium, isoerythritol and manganese.
- Selective media for Brucella are Albimi medium and Columbia agar.
- Serum Dextrose Agar, Potato infusion agar, Tryptose Soya Agar, TryptoseThiamine Agar are useful for primary isolation.

- The SDA enriched with bacitracin, cycloheximide, nalidixic acid, nystatin and polymyxin B is used as selective medium.
- After 3-5 days incubation on selective serum agar, pinpoint smooth, glistening, bluish, translucent colonies will appear.
- Strains of *B.ovis* and *B.canis* are always in the rough form. Others are initially smooth form and become rough on subsequent cultivation.
- Brucella is non haemolytic on blood agar. In liquid media growth is uniform and in old cultures a powdery or viscous deposit is formed.

BIOCHEMICAL PROPERTIES AND RESISTANCE

Biochemical properties

- Brucellae are catalase positive, oxidase positive (except *B.ovis* and *B.neotomae*), and urease +ve (except *B.ovis* and *B.melitensis*), reduce nitrate, IMViC, -,-,-,-. (indole, methyl red, voges proskauer and citrate utilization negative).

Resistance

- Readily killed by commonly used disinfectants and pasteurization.
- Brucellae are destroyed by heat at 60°C in 10mts and by 1% phenol in 15mts. They survive in soil and manure for several weeks.
- Remain viable for 10 days in refrigerated milk, one month in ice cream, 4 months in butter and many weeks in meat.
- *B.abortus* will survive for 41/2 hrs when exposed to direct sunlight, 4 days in urine, 5 days in cloth at room temperature and 75 days in an aborted fetus.

ANTIGENS AND BRUCELLA

Antigens

- The somatic antigens of brucellae contain two main antigenic determinants designated as A and M, which are present on the LPS protein complex.
- *Brucella abortus* contains more A antigen than M (20:1).
- *B.melitensis* has more M than A (20:1). *B.suis* has an intermediate pattern.
- *B.canis* and *B.ovis* are antigenically rough and do not possess the A and M antigens. Both possess a surface antigen R.
- Like many other Gram-negative bacteria, the change from S to R is associated with loss of virulence, a tendency to auto-agglutination and loss of antigen.
- The vaccine strain, *B.abortus* 19 is an intermediate mutant that, although low in virulence, is antigenic.
- Antigenic cross-reactions exist between brucellae with several bacteria like *Yersinia enterocolitica*, *Vibrio cholerae* and some strains of *E.coli* and *Salmonella* (Heterophile antigens).
- No exotoxins have been described. In addition to endotoxin, the surface cell wall carbohydrate is responsible for binding to B-lymphocytes and play a major role in pathogenesis.

Brucella biotyping

- Based on CO₂ requirement, H₂S production (*B.abortus* biotype 1,2,3,4 and *B.neotomae*), urease activity, growth in the presence of dyes (thionin, basic fuchsin), agglutination with monospecific sera (*B.canis* and *B.ovis* – agglutinate only

with anti R sera) and Phage typing, three biotypes have been recognized in *B.melitensis*, eight in *B.abortus* and four in *B.suis*.

- *B.abortus* biotype 3 is commonly present in India.

PATHOGENESIS

- The common routes of infection in animals and humans are via the mucous membrane of the digestive tract, genital tract and skin.
- **Veneral** transmission is main route for *B.ovis*. Less commonly infection may occur through inhalation or by conjunctiva.
- Organisms may penetrate the mucosa of nasal or oral cavities. Soon after entry into the host the brucellae are engulfed by phagocytic cells, in which they survive, multiply and are transported to the regional lymphnodes.
- After multiplication, the organism pass to the thoracic duct and then via blood stream to parenchymatous organs and other tissues such as joints, granulomatous foci develop in lymphatic tissues, liver, spleen, bone marrow and other locations. On occasion it will become abscess.
- Brucellosis is essentially disease of the sexually mature animal, the predilection sites being the reproductive tracts of males and females especially the pregnant uterus.
- Allantoic factors such as erythritol (Polyhydric OH), **steroid** hormones and other substances favour the growth of most brucellae.
- Erythritol is present in the placenta and male genital tract of cattle, sheep, goats and pigs but not humans.
- Erythritol does not stimulate the growth of *B.ovis* and inhibits *B.abortus* strain 19, the attenuated vaccine strain.
- A pyogranulomatous reaction occurs in affected placentae and abortion occurs from 6 month onwards.
- Note: Females usually abort only once, after which a degree of immunity develops, and the animals remains infected and large numbers of brucellae can be excreted in foetal fluids at subsequent parturitions. Permanent infertility may occur in male dogs infected with *B.canis*.
- Diseases caused by *Brucella* species

Species	H o s t	Dise ase
<i>B. abortus</i>	<ul style="list-style-type: none"> • Cattle • Sheep, Goat and Pig • Horses • Human 	<ul style="list-style-type: none"> • Abortion and orchitis (Bang's disease / Abortion storm) • Sporadic abortion • Poll evil and Fistulous withers • Undulant fever
<i>B.melitensis</i>	<ul style="list-style-type: none"> • Goat and Sheep • Cattle • Human 	<ul style="list-style-type: none"> • Abortion • Occasional abortion • Malta fever
<i>B.suis</i>	<ul style="list-style-type: none"> • Pig • Human 	<ul style="list-style-type: none"> • Abortion, orchitis, arthritis, spondylitis and infertility • Undulant fever
<i>B.ovis</i>	<ul style="list-style-type: none"> • Sheep 	<ul style="list-style-type: none"> • Epididymitis in rams and abortion in ewes

<i>B.canis</i>	<ul style="list-style-type: none"> • Dogs • Human 	<ul style="list-style-type: none"> • Abortion, epididymitis and permanent infertility in milk • Undulant fever
<i>B.neotomae</i>	<ul style="list-style-type: none"> • Desertwood rat 	<ul style="list-style-type: none"> • Non pathogenic

PATHOGENECITY

Symptoms

Cattle

- The disease in cattle is almost always caused by *B.abortus*.
- The incubation period is usually from 30 to 60 days.
- After bacteraemia the infection localizes in the placenta, if the animal is not pregnant, the infection localizes in udder (interstitial mastitis).
- In the bull, it localizes in the testicle and causes orchitis and epididymitis.
- Abortion at 6 months and retained placenta are the cardinal signs.

Sheep

- *B.melitensis* infections result in abortion at about 3rd or 4th month of pregnancy. Other signs are lameness and mastitis.

Swine

- Swine brucellosis is characterized by abortion, sterility, and birth of stillborn or weak pigs, focal abscesses in various organs, spondylitis and lameness.
- Abortion may occur at any time during gestation.

Dogs

- Expelled tissues and vaginal discharges of aborted bitches and the urine of infected males are primary sources of the infectious agent.
- The incubation period is 6 to 21 days. Infected bitches usually abort in the last trimester.
- Following abortion there is yellow brown to dark brown discharges that persist for 1 to 6 weeks.
- Epididymitis and testicular atrophy with decreased spermatogenesis are common in the male and may result in irreversible sterility.

Lesions

- The gravid bovine uterus infected with *B.abortus* develops a necrotic placentitis.
- The cotyledons become swollen, hyperaemic and surrounded by brownish exudates.
- The inter cotyledonary spaces are thickened and have a characteristic leathery appearance.
- Orchitis in bull results in abscess formation or areas of necrosis in the testicles, surrounded by fibrous tissues.

- *Brucella suis* causes placentitis, metritis in ewes and epididymitis and orchitis in boars.
- In horses, *B.abortus* infection associated with fistulous withers (a chronic inflammatory condition of the supraspinous bursa) and poll evil.

DIAGNOSIS

Specimens to be collected

- In abortion cases a full range of specimens should be collected and submitted for a differential diagnosis.
- A whole foetus should be sent, if feasible.
- Alternatively foetal stomach contents, any foetal lesions, cotyledons, uterine discharges, urine, coloustrum, paired serum samples and sections of cotyledons and foetal lesions in 10% formalin for histopathology. Semen and tissue from epididymis or testes from males could be examined.

Based on symptoms and lesions

Direct examination

- Modified ZN and Koster's stain are useful in demonstrating brucellae in smear from the placenta (cotyledons) in bovine abortion.
- Cells of the chorion are packed with organism, which stain red against a blue background.
- Organism can also be demonstrated directly in smears from vaginal mucous, semen and various tissues. Direct and indirect FAT is also used.

Based on isolation and identification

Based on biotyping

Animal inoculation

- Straus reaction: Orchitis in male guinea pigs.
- It is the most sensitive test for isolating pathogenic brucellae.
- It is highly useful if the material is badly contaminated or the numbers of organisms are very small.
- Guinea pigs are inoculated intra muscularly with 0.5 – 1 ml of tissue homogenate.
- Euthanise 6 wks after inoculation. Serum is taken for serology and the spleen, together with any abnormal tissue, is collected for bacteriological examination.

Immunological tests

- A wide range of immunological tests have been developed
 - Serum agglutination test/ Tube agglutination test (titre > 1:40 is +ve, titre <1:20 is doubtful). [Click here for visual](#)
 - ABRT/Milk ring test - [Click here for visual](#)
 - Coombs test: to detect incomplete antibodies
 - RBPT: Antigen consists of suspension of brucellae organisms stained with rose Bengal and adjust to pH 3.6

- Card test: Which uses only one serum dilution and stained antigen, is rapid, sensitive and used as a field screening test.
- Whey agglutination test
- CFT, AGID and ELISA
- Rivanol precipitation and Mercaptoethanol agglutination: to detect primary IgM antibodies
- PCR

CONTROL AND PREVENTION

- The immunity acquired from natural infection is not always sufficient to prevent reinfection.
- The general basis for elimination of brucellosis is testing and removal of reactors from the herd.
- Because the cattle will be less susceptible to reinfection, calf hood vaccination is recommended.
- The attenuated live vaccine (Strain 19 *B.abortus* biotype 1) is used in female calves 4 to 12 months of age.
- Because strain 19 cause infertility in some male calves, its use is restricted to females.
- The adjuvant bacterins eg: vaccine 45/20 is used as booster vaccine. *B.melitensis* Rev.1 live attenuated vaccine has been used with success to immunize rams against *B.ovis* infection.

