Brucella

Brucellosis is an infectious bacterial disease caused by members of the genus *Brucella.* It is a disease of worldwide importance and affects a number of animal species. *Brucella* are obligate parasites, requiring an animal host for maintenance. Infections tend to localize to the reticulo-endothelial system and genital tract with abortions in females and epididymitis and orchitis in males the most common clinical manifestations. Chronic infections are common.

The different *Brucella* species exhibit host preferences and vary in severity of the disease caused. Dye and phage susceptibility along with biochemical, cultural, and serologic characteristics are used to distinguish among species. The six *Brucella* species are *B. abortus, B. canis, B. melitensis, B. neotomae, B. ovis,* and *B. suis.*

Many species of Brucella are capable of causing disease in humans. Infections are chronic and debilitating.

A-DESCRIPTIVE FEATURES

-Morphology and Staining

Brucella are small, gram-negative coccobacilli measuring 0.6 to 1.5µm x 0.5 to 0.7 µm in size. Cells are fairly uniform and can easily be mistaken for cocci. They are typically arranged singly but also occur in pairs or clusters. No capsules, flagella, or spores are produced; however, an external envelope has been demonstrated by electron microscopy around *B. abortus, B. melitensis, and B. suis.* Brucella stain red with modified Ziehl-Neelsen stains.

-Cellular Structure and Composition

Brucella possess a typical gram-negative cell wall. Dominant surface antigens are located on the lipopolysaccharide. Specifically, the A and M antigens are found in varying concentrations among the different smooth *Brucella* species. The mol% G + C of DNA is 57. Plasmid DNA has not been demonstrated.

-Growth Characteristics

On initial isolation, colonies are not apparent until 3 to 5 days' incubation. Most colonies are detected by 10 to 14 days, but in some cases incubation for up to 21 days is required. Growth is best in an aerobic environment at 37°C. *Brucella ovis* and some biovars of *B. abortus* require an increased concentration of CO2. Enriched media with 5% serum is required by *B. abortus* biovar 2 and *B. ovis*. *Brucella* colonies have a characteristic bluish color when examined with obliquely transmitted light. Colonies have smooth or non smooth morphologies that are determined by the presence or absence, respectively, of the polysaccharide side chain in the lipopolysaccharide.

These morphologic variations are the result of spontaneous mutation and are influenced by specific growth factors. Smooth colonies are white, convex with an entire edge and have a creamy consistency. Non-smooth colonies have intermediate, rough, or mucoid forms. Rough colonies are dull yellow, opaque, and friable. They are difficult to suspend in solution and agglutinate spontaneously. The mucoid colonies are similar to the rough colonies except for having a glutinous texture.

-Resistance

Brucella survive freezing and thawing. Under proper environmental conditions, they survive for up to 4 months in milk, urine, water, and damp soil. Most disinfectants active against other gram-negative bacteria kill Brucella. Pasteurization effectively kills Brucella in milk.

-Diversity

The colony morphology of *Brucella* varies from rough to smooth forms. *Brucella abortus, B. melitensis, B. suis,* and *B. neotomae* are typically isolated in the smooth form but can develop rough forms on subsequent laboratory passage. *Brucella ovis* is always in a rough form. Isolates of *B. canis* have a mucoid appearance. In general, smooth strains of *Brucella* are more virulent than rough strains.

Variation in *CO2* requirement, H2S production, urease production, susceptibility to varying concentration of certain dyes (thionin and basic fuchsin), and susceptibility to bacteriophages account for diversity among species and biovars within species. The different species of *Brucella* vary in host preference and degree of virulence within and among animal species.

B-ECOLOGY

- Zoologic and Geographic Reservoirs

Brucella require an animal reservoir (obligate parasites). Host preference is exhibited by the different *Brucella* species. Survival time outside the host is variable and depends on temperature and moisture (colder weather extends survival time). Cattle are the preferential host for *B. abortus.* Other animals, including bison and camels are commonly infected. The different biovars of *B. abortus* have different geographic distributions. Biovars 1 and 2 have a worldwide distribution, while biovar 3 is predominantly found in India, Egypt, and Africa. Biovar 5 is most commonly encountered in Germany and the United Kingdom.

Swine are the preferential host for *B. suis*, *Brucella melitensis* infects goats and sheep worldwide. *Brucella ovis* infections are limited to sheep and *B. canis* infections are limited to dogs. Lastly, *Brucella neotomae* has only been found in the wood rat.

-Transmission

Brucella are disseminated by direct or indirect contact with infected animals. Ingestion is the most common route of entry, although exposure through the conjunctival and genital mucosa, skin, and respiratory routes occurs. The major source for exposure to *B. abortus* in cattle and *B. melitensis* in sheep and goats is through aborted fetuses, the placenta, and postabortion uterine fluids. Aborted tissue and fluids are also a common means for transmission of *B. suis and B. canis.* Genital infections in cattle routinely clear within 30 days after calving and cows are not considered infectious for other cattle after that time.

Ingestion of milk from infected cattle and goats is another source for infection of calves and kids. Direct transfer in utero has also been documented. Infections of the accessory sex glands of males allows for dissemination of organisms through the semen. Infections can occur in the accessory sex organs without testicular or epididymal lesions being present. Venereal transmission of *B. suis* in swine, *B. ovis* in sheep, and *B. canis* in dogs is common. Urine is another vehicle for disseminating *B. canis* to other dogs.

Insects may play a minor role in transmission and maintenance of infection in a herd. Face flies have been shown to take up and excrete *Brucella* in feces.

C-Pathogenesis

-Mechanisms

Following exposure, *Brucella* penetrate intact mucosal surfaces. In the alimentary tract, the epithelium covering the ileal Peyer's patches are a preferred site for entry. After penetrating mucosal

barriers, organisms may be engulfed by phagocytic cells. They are capable of surviving and multiplying inside macrophages by inhibiting phagolysosome fusion.

Following entry into the host, *Brucella* organisms, either free in the extracellular environment or in phagocytic cells, localize to regional lymph nodes. There they proliferate and infect other cells or are killed and the infection is terminated. Some cattle appear to be innately resistant to infection. This resistance is related to the macrophages' ability to contain the organisms. From the regional lymph nodes, *Brucella* disseminate hematogenously and localize in the reticuloendothelial system and reproductive tract.

There is preferential localization to the reproductive tract of the pregnant animals. Unknown factors in the gravid uterus, collectively referred to as *allantoic fluid factors,* stimulate the growth of *Brucella.* Erythritol, a fourcarbon alcohol, is considered to be one of these factors.

likely possibilities are that abortion results from;

1) interference with fetal circulation due to the existing placentitis,
2) the direct effect of endotoxin, and/or
3) fetal stress resulting from the inflammatory response in fetal tissue.

However, localization of *Brucella* in the reproductive tract of males, may be due to the presence of growth stimulating compounds.

-Pathology

There are grossly visible lesions in the placenta associated with *Brucella* abortions. Intercotyledonary thickening with a yellow gelatinous fluid is present. The cotyledons are frequently necrotic, yellow-gray in color, and covered with a thick brown exudates(*B. melitensis*
infections in goats being most severe). The aborted fetus is frequently edematous. Abomasal contents may be turbid and have a lemon-yellow color.

In males palpable enlargement of the epididymis, especially involving the tail portion, is common. Extragenital tract pathology includes lymphocytic endophthalmitis in dogs *(B. canis),* purulent or fibrinopurulent synovitis in swine *(B. suis),* osteomyelitis in dogs and swine *(B. canis* and *B. suis),* necrotizing and purulent bursitis in horses *(B. abortus),* and hygroma development in cattle *(B. abortus).*

-Disease Patterns

Abortion in cattle commonly occurs in the fifth month of gestation or later. Retained placenta is a possible sequela. Females usually abort only once, presumably due to acquired immunity.

Brucella melitensis infections in goats and sheep are similar to *B. abortus* in cattle except that acute mastitis develops in goats infected with *B. melitensis.* The mastitis in goats presents with palpable nodules in the udder and milk that is clotted and watery. Abortions in swine can occur at any time in gestation and are related to time of exposure. Abortions in dogs due to *B. canis* occur around 50 days of gestation. *Brucella ovis* infections in sheep only rarely result in abortions in ewes.

In males, epididymitis and orchitis are the most common presenting signs. Lesions are usually unilateral but may be bilateral.

-Epidemiology

Humans acquire infections by handling tissues containing *Brucella* organisms. *Brucella melitensis* is considered the most virulent species for humans followed by *B. suis, B. abortus,* and *B. canis. Brucella ovis* and *B. neotomae* do not infect humans. Infected animals also shed organisms in the milk. Raw milk or raw milk products of bovine or caprine origin are ready sources for infections in humans.

D-LABORATORY DIAGNOSIS

-Specimens

Appropriate samples for diagnosis of brucellosis depend on the animal species affected, species of *Brucella* involved, and clinical presentation. Abscess material, semen, and vaginal fluids associated with recent abortions are useful for recovering organisms antemortem. Milk samples from cattle and goats are used in antemortem isolation attempts and for immunodiagnostic
evaluation. In dogs, blood cultures are useful for isolation of *B. canis* because of the prolonged bacteremia that occurs. Serum is used for serologic evaluation.

Samples collected at necropsy should include spleen, liver, udder, and multiple lymph nodes, including the supramammary, retropharyngeal, internal iliac, lumbar, and mesenteric lymph nodes. The supramammary lymph node is superior to other lymph nodes for isolating *Brucella*
from dairy cattle. Abomasal fluid and lungs of the aborted fetus and the placenta are the preferred specimens in the case of abortion. In males the epididymis, testicle, and accessory sex organs are examined.

-Direct Examination

Modified Ziehl-Neelsen stain of fetal stomach contents from an aborted fetus and the placenta reveal large numbers of gramnegative coccobacilli.

-Isolation

Tissues are cultured directly on solid media. Milk, both the cream layer and sediment,
if the centrifugation technique is used, should be plated on solid media. Commonly used media include serum dextrose, tryptose, and brucella agars. If contamination is likely to be a problem, isolation attempts should be made using media containing antibiotics with or without the incorporation of ethyl violet (1:800,000). Cultures should be incubated at 37°C in 10% *CO2* for a minimum of 10 days and up to 21 days in highly suspicious cases. Animal inoculation is the most sensitive method for detection of *Brucella* and is sometimes necessary when very low numbers of organisms are present. Guinea pigs are the most sensitive laboratory animals for this purpose.

Two guinea pigs are inoculated and at 3- and 6-weeks post inoculation an animal is sacrificed. Serum is examined for antibodies and tissues are cultured for organisms.

-Identification

Preliminary identification of *Brucella* species requires demonstrating colonies of gram-negative coccobacilli that are nonhemolytic, catalase positive, and oxidase positive (except for *B. avis* and some strains of *B. abortus).* Most species, except *B. avis,* are strongly urease positive. Glucose and lactose are not fermented by any of the species. Agglutination in unadsorbed antismooth *Brucella* serum helps in preliminary identification of smooth strains.

A fluorescent antibody test is used for rapid identification. Urease production, *CO2* requirement, H2S production, oxidation of metabolic substrates, agglutination in mono specific antisera, growth in

the presence of varying concentration of thionin, and basic fuchsin and phage typing are used to determine species and biovars within species.

-Immunodiagnosis

Antibody detection is commonly used for diagnosing brucellosis and in control programs. Samples tested include blood, milk, and occasionally semen. A number of immunodiagnostic tests have been developed for cattle. Individual blood samples can be tested by tube agglutination, plate agglutination, rose bengal plate, or card tests. Other tests include the buffered plate agglutination assay, rivanol agglutination, complement fixation, and enzyme-linked immunosorbent assay (ELISA).

Milk is screened with the Brucella milk ring test, which identifies specific antibodies in milk. Stained *Brucella* antigen is added to milk. If antibodies are present, agglutinated antigen is buoyed يرتفع to the top by the rising cream and a purple ring develops at the top of the tube.

-Nonculture Detection Methods

A number of nonculture methods, including PCR, immunoperoxidase staining, DNA probes, and coagglutination, have been described for detection of *Brucella* in tissues and fluids.

E-TREATMENT

As a general rule, treatment of infected livestock is not attempted because of the high treatment failure rate, cost, and potential problems related to maintaining infected animals in the face of ongoing eradication programs.

F-CONTROL AND PREVENTION

Approaches used to control brucellosis include:

1) immunization alone,
2) testing and removal of infected animals in conjunction with an immunization program, and
3) testing and removal of infected animals without immunization.