Brucella

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General Concepts

Clinical Manifestations

Brucellosis is a severe acute febrile disease caused by bacteria of the genus Brucella. Relapses are not uncommon; focal lesions may occur in bones, joints, genitourinary tract, and other sites. Hypersensitivity reactions can follow occupational exposure. Infection may be subclinical. Chronic infections may occur.

Structure

Brucellae are Gram-negative coccobacilli; non-spore-forming and non-motile; aerobic, but may need added CO2.

Classification and Antigenic Types

Three species (B melitensis, B abortus, B suis) are important human pathogens; B canis is of lesser importance. Species are differentiated by production of urease and H2S, dye sensitivity, cell wall antigens and phage sensitivity. The major species are divided into multiple biovars.

Pathogenesis

Portals of entry are the mouth, conjunctivae, respiratory tract and abraded skin. Organisms spread, possibly in mononuclear phagocytes, to reticuloendothelial sites. Small granulomas reveal a mononuclear response; hypersensitivity is a major factor.

Host Defenses

Effective host defense depends mainly upon cell-mediated immunity.

Epidemiology

Brucellosis is a zoonosis, acquired from handling of infected animals or consuming contaminated milk or milk products. Exposure is frequently occupational. The disease is
now uncommon in the United States and Britain but common in the Mediterranean and Arabian Gulf regions, Latin America, Africa, and parts of Asia.

**Diagnosis**

Diagnosis can be made clinically if there is a history of exposure. Blood cultures may be positive in early disease but serology is mainstay of diagnosis. Interpretation is complicated by subclinical infections and persistent levels of antibody.

**Control**

Brucellosis is prevented by pasteurizing milk, eradicating infection from herds and flocks, and observing safety precautions (protective clothing and laboratory containment). The disease is treated with doxycycline, streptomycin and rifampin.

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**INTRODUCTION**

Bacteria of the genus Brucella cause disease primarily in domestic, feral and some wild animals and most are also pathogenic for humans. In animals, brucellae typically affect the reproductive organs, and abortion is often the only sign of the disorder. Human brucellosis is either an acute febrile disease or a persistent disease with a wide variety of symptoms. It is a true zoonosis in that virtually all human infections are acquired from animals. The disease is controlled by the routine practice of pasteurizing milk and milk products, as well as by comprehensive campaigns to eradicate the disease by destroying domestic animals which exhibit positive serologic reactions to brucellae. Vaccines providing some protection to cattle, sheep and goats are available.

**Clinical Manifestations**

The presentation of brucellosis is characteristically variable. The incubation period is often difficult to determine but is usually from 2 to 4 weeks. The onset may be insidious or abrupt. Subclinical infection is common.

In the simplest case, the onset is influenzalike with fever reaching 38 to 40oC. Limb and back pains are unusually severe, however, and sweating and fatigue are marked. The leukocyte count tends to be normal or reduced, with a relative lymphocytosis. On physical examination, splenomegaly may be the only finding. If the disease is not treated, the symptoms may continue for 2 to 4 weeks. Many patients will then recover spontaneously but others may suffer a series of exacerbations. These may produce an undulant fever in which the intensity of fever and symptoms recur and recede at about 10 day intervals. Anemia is often a feature. True relapses may occur months after the initial episode, even after apparently successful treatment.
Most affected persons recover entirely within 3 to 12 months but some will develop complications marked by involvement of various organs, and a few may enter an ill-defined chronic syndrome. Complications include arthritis, often sacroiliitis, and spondylitis (in about 10 percent of cases), central nervous system effects including meningitis (in about 5%), uveitis and, occasionally, epididymoorchitis. In contrast to animals, abortion is not a feature of brucellosis in pregnant women. Hypersensitivity reactions, which may mimic the symptoms of an infection, may occur in individuals who are exposed to infective material after previous, even subclinical, infection.

Structure

Brucellae are Gram-negative coccobacilli (short rods) measuring about 0.6 to 1.5 µm by 0.5-0.7 µm. They are non-sporing and lack capsules or flagella and, therefore, are non-motile. The outer cell membrane closely resembles that of other Gram-negative bacilli with a dominant lipopolysaccharide (LPS) component and three main groups of proteins. The guanine-plus-cytosine content of the DNA is 55-58 moles/cm. No Brucella species has been found to harbor plasmids naturally although they readily accept broad-host-range plasmids.

The metabolism of the brucellae is mainly oxidative and they show little action on carbohydrates in conventional media. They are aerobes but some species require an atmosphere with added CO₂ (5-10 percent). Multiplication is slow at the optimum temperature of 37°C and enriched medium is needed to support adequate growth.

Brucella colonies become visible on suitable solid media in 2-3 days. The colonies of smooth strains are small, round and convex but dissociation, with loss of the O chains of the LPS, occurs readily to form rough or mucoid variants. These latter forms are natural in B canis and B ovis as the LPS of these lack O chains.

Classification and Antigenic Types

Distinguishing features of the six species of Brucella and their preferred hosts are shown in Table 28-1. B abortus, B melitensis and B suis are serious pathogens in humans, B canis causes mild disease and the other two species have not affected humans.
A culture can be identified as belonging to the genus Brucella on the basis of colonial morphology, staining and slide agglutination with anti-Brucella serum, smooth or rough. Further classification is best done in a specialized laboratory. Identification to species level may be done by the procedures shown in Table 28-1. Further differentiation to biovars may be useful and is illustrated in Table 28-2. As a further refinement, tests for the oxidative metabolism of certain aminoacids and carbohydrates have been devised. Modern DNA hybridization tests, however, show that the currently named species show a high degree of homology and suggest that the genus could be appropriately reclassified as having a single species.
The application of techniques of molecular biology have allowed the cloning and characterization of several genes coding for outer membrane proteins, the use of PCR to identify the presence of brucellar DNA at genus and species level and the demonstration of species specific patterns of restriction fragment length polymorphism. It is predictable that this work will be extended to improve diagnostic tests and even vaccine development.

Two different O chains in brucellae occur in the LPS of the brucellae with smooth colonies. These are called A and M, nominally indicating abortus and melitensis antigens. (‘Nominally’, because some abortus biovars carry the M antigen and some common melitensis biovars the A antigen.) Both O chains have been shown to be homopolymers of 4,6-dideoxy-4-formamido-d-mannopyranose; they differ only in that in the A chain the sugar molecules are always linked 2-1 whereas the M chain has every fifth junction a 3-1 linkage. In routine serology, smooth species of brucellae cross-react almost completely with each other, but not with rough species and vice versa. Monospecific polyclonal sera reacting only to A or M antigens are prepared by cross absorption and monoclonal antibodies specific for A and M antigens are now available, indicating that there is at least one unique epitope on each type of chain.

**Pathogenesis**

Brucellae are facultative intracellular parasites, multiplying mainly in monocyte-macrophage cells. This characteristic dominates the pathology, clinical manifestations and therapy of the disease.
The organisms may gain entry into the body through a variety of portals (Fig. 28-1). Because the infection is systemic it is often not possible to determine which portal was involved in a particular case. Oral entry, by ingestion of contaminated animal products (often raw milk or its derivatives) or by contact with contaminated fingers, probably represents the most common route of infection even though this portal may not be the most vulnerable one. Inhalation of aerosols containing the bacteria, or aerosol contamination of the conjunctivae, is another route. Inhalation probably underlies some industrial outbreaks. Percutaneous infection through skin abrasions or by accidental inoculation has frequently been demonstrated.

![Figure 28-1 Portals of entry for Brucella species.](image)

Brucella species differ markedly in their capacity to cause invasive human disease. *Brucella melitensis* is the most pathogenic; *B abortus* is associated with less frequent infection and a greater proportion of subclinical cases. The virulence of *B suis* strains for humans varies but is generally intermediate.

Animal studies suggest that invading brucellae are rapidly phagocytosed by polymorphonuclear leukocytes. Brucellae are frequently able to survive and multiply in these cells because they inhibit the bactericidal myeloperoxidase- peroxide-halide system by releasing 5'-guanosine and adenine. Early in infection, macrophages are also relatively ineffectual in killing the intracellular brucellae (Fig. 28-2). In systemic spread, it is not clear whether the bacteria are transported within neutrophils and macrophages or in the blood stream outside cells but organisms may disseminate widely from regional lymphoid tissue appropriate to the portal of entry and may localize in certain target organs such as lymph nodes, spleen, liver, bone marrow, and (especially in animals) the reproductive organs. The presence of meso-erythritol in the testicles and
seminal vesicles of bulls, rams, goats, and boars and in the products of conception in pregnant ruminants and pigs stimulates enormous multiplication of brucellae. Erythritol represents a potent localizing factor in the relevant species, but is absent in humans.

Figure 28-2 Spread of Brucella in the body.

In humans, the tissue lesions produced by Brucella species consist of minute granulomas that are composed of epithelioid cells, polymorphonuclear leukocytes, lymphocytes and some giant cells. In cases of infection with B melitensis these granulomas are particularly small although the toxemia associated with this organism is great. Necrosis is not common, and abscesses do not form, except in B suis infection. The fact that humans rapidly develop hypersensitivity to brucellar antigens suggests that many of the symptoms of human brucellosis result from the reaction of the host defenses.

Host Defenses

The specific host defenses against brucellae resemble those against other intracellular bacteria and are both humoral (antibody-mediated) and cell-mediated. Passively administered monoclonal antibody directed against LPS has been shown to reduce the numbers of brucellae surviving in the spleens and livers of experimental mice, indicating a role for antibody in protection. However, the principal component in defense against brucellae is cell-mediated. Macrophages have been shown to process brucellar antigen and present this to T lymphocytes which produce lymphokines. These agents, of which interferon is the most active in this context, activate the formerly ineffective macrophages to greater bactericidal potency. Depletion of gamma interferon makes experimental animals vulnerable to infection. T cell-derived lymphokines are
also involved in attracting cells to the foci of infection. This leads to granuloma formation. While this contributes to the pathology, it also delivers the activated macrophages to the site where they are needed. This inflammatory response is enhanced by cytokines, such as the colony-stimulating factors, tumor necrosis factor and interleukin-1, produced by a number of cell types.

Mice which have survived brucellosis are protected against further challenge, and there is clinical evidence that complete recovery from a natural infection is associated with at least a degree of residual resistance in humans.

**Epidemiology**

The reservoirs of brucellosis are various wild, feral and (particularly) domestic animals (Fig. 28-3). In ruminants, enormous numbers of bacteria are shed widely from infected products of conception, whether aborted or born at term. Brucellae frequently invade the mammary gland of infected ruminants. This organ can even be directly infected by any of the major species of Brucella, for example by contaminated hands. This may allow milk cows, for example, to excrete large numbers of organisms not only B abortus, but B melitensis or B suis and such milk spread has resulted in extensive outbreaks of brucellosis.

![Figure 28-3 Sources of Brucella infection.](image)

Brucella melitensis in sheep and goats represents, by far, the most important source of brucellosis in humans. This species of Brucella is not enzootic in the United States, Canada, northern Europe, Australasia or South East Asia. It is prevalent in Latin America, the Mediterranean area, Central Asia and, especially, in the countries around
the Arabian Gulf. Humans are principally infected by the handling of parturient animals and the consumption of raw milk and milk products, especially fresh soft cheeses. In many cases the vehicle of infection is uncertain and bacteria-laden dust is suspected.

Brucella suis occurs in most areas in which pigs are kept. It affects both sexes of swine causing infertility, abortion, orchitis and lesions of bones and joints. The prevalence is generally low except in parts of South America and South East Asia. In both south eastern United States and in Australia, particularly Queensland, populations of feral swine are heavily infected. Apart from the rare cases in which cows' milk is infected, infections with B suis in humans occur in people handling pigs on farms and during slaughtering and processing - including the hunting of feral swine. While an eradication campaign in the United States has made much headway, abattoir outbreaks still have occurred recently.

Bovine brucellosis, caused by B abortus, has been eradicated from Canada, Japan, northern Europe and Australasia. Cases in humans tend to be sporadic and often stem from occupational exposure. Infection can be acquired by drinking unpasteurized milk, but this a relatively inefficient mode of spread, while abattoir workers can be significantly exposed and veterinarians are doubly at risk from attending parturient cattle and from accidental inoculation with live vaccine.

Cases of B canis infection in man have tended to occur only in dog handlers. Close, frequent contact seems to be necessary for transmission.

Cases of brucellosis have continued to present in some regions from which brucellosis has been effectively eradicated. These are caused, usually by B melitensis, in travelers to popular tourist destinations, like Mexico and the Mediterranean region, in which this organism is highly prevalent, and by the importation of infected dairy products. Although person to person spread is quite exceptional, the species of principal importance are all potent causes of laboratory infections. Hence stringent safety measures, including adequate containment to reduce the hazard of aerosol spread, are essential. The situation has been aggravated by the failure of certain popular laboratory kits to identify Brucella spp appropriately.

**Diagnosis**

The diagnosis of brucellosis is primarily dependent on clinical suspicion allied with the taking of an adequate history of possible exposure - including during travel. Presentation can, however, be highly atypical and focal lesions may present decades after exposure.

Unequivocal diagnosis requires isolation of the organism. Blood culture is the method of choice but specimens need to be obtained early in the disease and cultures may need to be incubated for up to four weeks. Even so, failure to grow the organism is common, especially in cases of B abortus infection, and isolation rates of only 20-50% are reported even from experienced laboratories. Modern commercial systems are hampered by the small amount of CO2 produced during growth. Culture from bone marrow and from presenting foci may be successful. Presumptive identification of cultures from morphology and slide agglutination with specific antiserum should be followed by
further work in a reference facility. Molecular techniques for typing are being developed.

Figure 28-4 Diagnosis of brucellosis.

Serology remains the mainstay of laboratory diagnosis, but the interpretation of results is fraught with difficulties. The large number of techniques in use is evidence of the problems. The standard serum agglutination test (SAT) has been augmented by the modified Coombs' (antiglobulin) technique and the use of 2-mercaptoethanol to separate the actions of specific IgG and IgM. These classical methods may, in time, be supplanted by EIA (enzyme immunoassay) tests, designed to differentiate between specific IgM and IgG antibodies. While the SAT titers commonly decline after recovery from infection and antiglobulin test levels are maintained much longer, the IgM antibody that is commonly measured by the SAT does not fall away as regularly as in some infections. Nevertheless, persisting levels of antibody may indicate a remaining focus of infection and specific IgG levels rise again with a true relapse.

Further, because cases often are investigated late in their course, rising titers are frequently missed; the variability of individual responses and the frequency of subclinical infections make the interpretation of single high titers subject to error. All serologic tests have to be interpreted with caution in the light of clinical data and in the context of the local prevalence of brucellosis. Moreover, serum from persons with tularemia may show cross-reactions with Brucella antigen.

The diagnosis of the chronic brucellosis syndrome, without specific localization, is often very unsatisfactory. When cultures are negative and the results of serologic tests are equivocal a confident diagnosis is often impossible.
Control

Individuals who are occupationally exposed can be protected to some extent by wearing impermeable clothing, rubber boots, gloves and face masks and by practicing good personal hygiene. Pasteurization of milk for drinking and for incorporation into other dairy products is effective in protecting consumers. No widely accepted vaccines for humans have been developed but progress in the understanding of brucellar epitopes and of immunology could change this.

However, eradication of brucellosis from domestic animals reduces dramatically the threat to humans and has been successful in several countries. In eradication campaigns, the level of enzootic disease can first be reduced by intensive use of live, attenuated vaccines (B abortus strain 19 in cattle, B melitensis strain Rev. 1 for sheep and goats) particularly in immature animals. Thereafter, the emphasis shifts to the detection of infected herds (by skin tests in sheep; serologic tests on milk or blood samples taken at sale or slaughter in cattle) and individual animals (by serologic tests) and to the elimination of the latter by slaughter.

Humans are treated with combinations of antibiotics for from 4 to 6 weeks. Doxycycline and rifampin form the basis, with cotrimoxazole replacing doxycycline in children, but fewer relapses are reported with regimens including two weeks of daily streptomycin. Azithromycin has shown promising results in experimental models.

REFERENCES


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