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Brucellosis: Diseases, clinical signs, diagnosis, treatment and control: Review

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Abstract
Brucellosis is a zoonotic infection transmitted from animals to humans by the ingestion of infected food products, direct contact with infected animal or inhalation of aerosols. *Brucella* is a facultative intracellular pathogen that has the ability to survive and multiply in the phagocytes and cause abortion in cattle and undulant fever in humans. *Brucella* spp particularly *B. melitensis*, *B. abortus*, and *B. suis* represent a significant public health concern. At present, *B. melitensis* is the principle cause of human brucellosis in India. Late aborted fetuses, may be born alive but either die shortly after birth or are weak, unthrifty, and at risk of succumbing to calf diarrhoea. Most cows that abort have RFM. The placenta appears dry, thickened, cracked, and covered by a yellowish exudate in the inter cotyledonary areas. Efforts are directed at detection and prevention, because no practical treatment is available. Eventual eradication depends on testing and eliminating reactors. The disease has been eradicated from many individual herds and areas by this method. Herds must be tested at regular intervals until two or three successive tests are negative vaccination of calves with *B abortus* Strain 19 or RB51 increases resistance to infection.

Keywords: brucellosis, zoonotic, *vaccination*

Introduction
Brucellosis is a chronic infectious disease caused by small, non-motile, non-sporing, gram-negative, facultative intracellular coccobaccilli of the genus *Brucella*. Brucellosis is a zoonotic infection transmitted from animals to humans by the ingestion of infected food products, direct contact with an infected animal or inhalation of aerosols. In many countries have eradicated Brucellosis in cattle, caused by brucellosis in some areas. *Brucella melitensis* has emerged as a cause of infection in sheep and goats. *Brucella suis* is also emerging as an agent of infection in cattle, thus extending its opportunities to infect humans. *Brucella* is a facultative intracellular pathogen that has the ability to survive and multiply in the phagocytes and cause abortion in cattle. Breucella cause undulant fever in humans. It is zoonotic in nature. *Brucella* spp particularly *B. melitensis*, *B. abortus*, and *B. suis* represent a signifificant public health concern. Bovine brucellosis is usually caused by *Brucella abortus*. *Brucella melitensis*, transmitted from sheep and goats, can also cause disease in cattle. It is highly pathogenic in nature and caused abortion storm. Although *B. suis*has been isolated from cattle in contact with infected pigs, it does not appear to cause disease in cattle [8]. The recent isolation of distinctive strains of Brucella from marine mammals has extended its ecologic range. Molecular genetic studies have demonstrated phylogenetic affiliation to *Agrobacterium*, *Phyllo bacterium*, *Ochrobactrum*, and *Rhizobium*. At present, *B. melitensis*is the principle cause of human brucellosis in India. Molecular studies have now highlighted the pathogenesis of *Brucella*, for the development of newer diagnostic tools that will be useful in developing countries where brucellosis is a common, but often a neglected disease.

History
A type of fever characterized by fairly regular remissions or intermissions has been recognized along the Mediterranean littoral since the time of Hippocrates in 450 B.C. It is noteworthy that long ago in his publication Epidemics, Hippocrates described brucellosis-type syndromes in humans living in the Mediterranean littoral. Captain David Bruce (1887) a Scottish physician, reported small coccal organisms in the stained sections of spleen of a fatally infected soldier. This disease was fully elucidated by Sir David Bruce, Hughes, and Zammit working in Malta [10]. Greek physician, Themistokles Zammit identify milk products of goats as the source of infection for military troops on the island of Malta.

From the time of the Roman era, organisms resembling *Brucella*e have been detected in carbonized cheese. The first case of human brucellosis was recognized in 1897 in a US army officer [8] The organism derived its species name from Melita (honey), the roman name for the Isle of Malta. Even after more than a century of extensive research, Brucella spp. are still serious animal pathogens that cause brucellosis, a zoonosis that results in substantial economic losses, human morbidity, and perpetuates poverty worldwide [23]. Human infection is mainly acquired by the oral, respiratory, or conjunctival routes, but ingestion of raw milk products constitutes the main risk to the general public in countries in which the disease is endemic. There is an occupational risk to veterinarians, abattoir workers, and farmers who handle infected animals/carcasses and aborted fetuses or placenta [17].

Bang discovered *B. abortus*, the causative agent of abortion in cattle and of brucellosis (undulant fever) in human beings. Despite being endemic in many developing countries, brucellosis is under-diagnosed and under-reported. [15] Brucellosis endemically present in India, since its first report from Indian Veterinary Research Institute (IVRI), Mukteswar [2]. Both humoral and cellular immune responses are involved in *Brucella* infection, though magnitude varies with virulence of organism and immune status of animals

Epidemiology

B. abortusis found worldwide in cattle-raising regions, except for Japan, Canada, Scandinavia, Luxembourg, the Netherlands, some central European countries, Australia, New Zealand, and Israel, where it has been eradicated [22]. The UK is considered to be free of the disease, and most of the states of the mainland US are also free [31]. Taxonomically, *Brucella* are classified as alph-Proteobacteria and subdivided into 6 species [21] These 6 species under the genus *Brucella* comprise: *Brucella melitensis, B. abortus, B. suis, B. ovis, B. canis* and *B. neotomae* [13].

The genome of *Brucella abortus* biotype 1 and 4 and *Brucella suis* biotype 1 are found quite similar to *Brucella melitensis* [17]. The principal source of infection is aborting cows in which the fetus, placenta, fetal fluids, and milk are all heavily contaminated. Ingestion of contaminated pasture, bedding, food, or water, or licking an aborted fetus, infected afterbirth, or genital exudate from a recently aborted cow are common means by which transmission occurs.

Brucella spp. infects humans as an incidental host. Human infection usually results from direct contact with tissues or blood from infected animals or by consumption of contaminated animal products, including unpasteurized milk and cheeses. The low number of virulent organisms required for infection combined with the capacity for aerosolization renders *Brucella* spp. as category B pathogens and potential agents for bioterrorism. With an infectious dose of 10 to 100 organisms, the calculated financial risk of such an attack is second only to anthrax and tularemia.

Pathogenesis

Brucella spp are facultative intracellular bacteria that have the ability to avoid the killing mechanism and proliferate within the macrophages, similar to other intracellular pathogens. To be a successful infectious agent, *Brucella* requires four steps: adherence, invasion, establishment, and dissemination within the host. Brucellae display strong tissue tropism and replicate within vacuoles of macrophages, dendritic cells (DCs), and placental trophoblasts. Brucella survive and replicate inside professional phagocytic cells, evade and modulate the host immune response, and disseminate to preferred tissues through cellular tropism, for example, placental trophoblasts in pregnant females, fetal lung, reticuloendothelial system, and reproductive tract [1]. *In vitro* studies were used as models to understand adhesion, internalization, intracellular trafficking, survival, and replication of Brucella in susceptible hosts. Thus, after attachment to the surface of mucosal epithelial cells, Brucella induces a zipper-like mechanism for internalization [26].

Once translocated through the epithelium, Brucella are engulfed by mucosal phagocytic cells in which <10% of phagocytized bacteria survive an adaptation period. To delay being recognized by the immune system and initiating an immune response, *Brucella reduce*, modify, or cloak their pathogen-associated molecular patterns [3]. Inside mononuclear phagocytic cells, Brucella reside in a special vacuole (Brucella-containing vacuole, BCV), modify intracellular trafficking, and transform the vacuole into a replicative compartment or brucellosome [1].

Initially, the pathogen undergoes quantitatively reduced gene expression and protein synthesis involved in anabolic metabolism while increasing amino acid catabolism, switching to alternative energy sources, and altering respiration to adapt to low oxygen tension [19]. Brucella has several clever strategies to establish and maintain a chronic infection, including inhibition of apoptosis of infected mononuclear cells, reduced antigen presentation, and reduced activation of naive T cells [4]. Brucellae evade intracellular destruction by restricting fusion of the BCV (brucella containing vacuole) with the lysosomal compartment. The organism colonises the udder and supramammary lymph nodes of non-pregnant animals. In pregnant animals, production of erythritol within the placenta allows rapid multiplication of the bacteria, leading to endometritis, infection of cotyledons, and placentitis. The outer membrane contains Lipopolysaccharide (LPS), which is the major virulence factor of *Brucella*. It possesses a peculiar non classical LPS as compared to the classical LPS from Enterobacteria, such as *Escherichia. col*i [25].

Clinical signs

The main sign of brucellosis is abortion, which occurs mainly in the second half of gestation. Late aborted fetuses, may be born alive but either die shortly after birth or are weak, unthrifty, and at risk of succumbing to calf diarrhoea. Most cows that abort have RFM. The placenta appears dry, thickened, cracked, and covered by a yellowish exudate in the intercotyledonary areas. Cotyledons appear necrotic and may also be covered with anexudates. In chronic condition joint hygroma may develop.

In male animals, orchitis and epididymitis are the manifestations of chronic infection [20]. During orchitis the testicles gradually enlarge. Areas of dry necrosis develop and become encapsulated by fibrinous tissue which eventually contracts, often leaving the testicles small erthan normal. In other cases, it may soften with the production of a soft fluctuating lesion containing thin pus like pea soup. Cases of abortion in infected herd are less in chronic infection but infected animal continue to act as carrier posing threat to the herd [9]. Localization of brucellae within the female and male reproductive tracts accounts for the principal clinical symptoms of infection, *viz*. abortion and male infertility [16]. In case of lungs, pneumonia is there which is usually of a

broncho-pneumonia type. Cobbler stone lesions on lungs are indicative of brucellosis [12].

Diagnosis

Diagnosis is based upon the isolation of *B. abortus* from abortion material, milk, or necropsy material. In addition or as an alternative, specific cell-mediated or serological responses to *Brucella* antigens can be demonstrated [11]. The organism can be identified in stained smears prepared from suspected contaminated material, either using a modified Koster and Ziehl-Neelsen method or a fluorescent antibody technique [6]. Demonstration of acid fast organisms provides a presumptive diagnosis, but *Chalmydia* or *Coxiella* can be mistaken for *B. abortus* in stained smears, and the sensitivity is low in milk. The organism can be isolated by microbial culture from the fetal stomach of an abortus, from fresh afterbirth, or from uterine exudate. LPS smooth chains producing the greatest immunological responses in various hosts which is the basis of serological tests. The major diagnostic problem is due to the similarity of the O-antigenic side chain of LPS of *Brucella* and other organisms like *Yersinia enterocolitica* O: 9, *Vibrio. cholerae, Esherichia. coli*: 157, and *Francisella. tularensis.* (Blood culture is the gold standard in the diagnosis of bacterial infections including brucellosis, but this method is successful in only 40 – 70% of the cases. Bone marrow cultures may provide higher sensitivity, yield faster culture times, and may also be superior to blood culture, when evaluating patients with previous antibiotic use. *Brucella* can also be cultured from pus, tissue, cerebrospinal fluid (CSF), and pleural / joint / ascitic fluid.

Serodiagnosis

In the absence of culture facilitates the diagnosis of brucellosis relies on agglutination tests, such as:

The Rose Bengal test, serum agglutination test, the antiglobulin or Coombs test, complement fixation test, and the recently introduced immunocapture test. Coombs test that detects incomplete antibodies and immunocapture-agglutination tests show good performances with higher sensitivity and specificity in the diagnosis of brucellosis.

The Rose Bengal test is used as a screening test and positive results are confirmed by the serum agglutination Tests [27]. Diagnosis at the herd level as part of eradication schemes haslargely relied upon serological tests upon biological materials such as milk, serum, vaginal mucus, and semen. The rose Bengal plate test, which was introduced into the UK in 1970 as the main initial screening test of serum samples in the brucellosis eradication scheme [7].

The plate agglutination test, which was rated highest, in terms of sensitivity and specificity. It was the conventional tests and is better than either rose Bengal or complement fixation tests [14]. The milk ring test, which detects *Brucella* antibodies in milk, is very useful in screening the presence of brucellosis in herds by collecting bulk milk samples or in individual animals [25]. Some ELISAs carried out to differentiation between vaccinated and infected animals test [30]. CFT persist at diagnostically significant levels. The CFT is also more effective than the SAT in differentiating titres arising from infection from vaccination. In calves vaccinated with Strain 19, titres detected by the CFT become negative in most cases by 6 months after vaccination, whereas an 18-month period is required for the SAT. SAT titers above 1 : 160 are considered diagnostic in conjunction with a compatible clinical presentation, however, in endemic areas the titer of 1 : 320 is taken as the cut off.

Coomb's test is the most suitable and sensitive test for confirmation in relapsing patients with persisting disease PCR and other molecular methods for the diagnosis of *Brucella* species now appear to be sufficiently rapid, simple, and robust to provide species–specific information and to be useful for the epidemiological study of brucellosis. Enzyme linked immunosorbant assay (ELISA) has become increasingly popular, as well as a standardized assay for brucellosis. It measures IgG, IgM, and IgA, which allows a better interpretation of the clinical situation.

As the diagnosis of *Brucella* is based on the detection of antibodies against smooth LPS, the cut-off value needs to be adjusted, to optimize the specificity when used in endemic areas [28].

Several genus-specific PCR systems using primer pairs that target 16SRNA sequences and genes of different outer membrane proteins have been developed (Queipo- Ortuno and co-workers found 100% sensitivity and 98.3% specificity by using a B4 / B5 primer and amplifying a 223-bp fragment of the *bcsp31* gene compared with 70% constituents of blood culture. Incorporation of a robust DNA extraction method, such as the diatom-guanidinium isothiocyanate method, effectively removes the inhibitors commonly present in a variety of clinical specimens and may improve the sensitivity and reproducibility.

Control

The Food and Agriculture Organization recommends the following sequence of action for eradication of brucellosis from a nation or region -

Phase 1: High or unknown prevalence, with no control programme

The first step is to identify the prevalence and distribution of the infection through programmes such as investigation of abortions and surveys of cattle on farm and in markets or at slaughter.

Phase 2: Mass vaccination

In the UK the first mass vaccination was undertaken with vaccines prepared from killed cultures of McEwan's *B. abortus*S45/20. This was later replaced with strain S19, a smooth variant of a strain of *B. abortus* of reduced virulence but of high antigenic quality. Strain RB51 (a rough strain) is also being used for vaccination; its advantage over S19 is that it is less likely to cross-react with serological tests for virulent strains. Vaccination should be supported by checks on and off farm that animals are seropositive.

Phase 3: Test and removal, segregation, or slaughter

Herds are tested for animals that are seropositive to virulent strains; these animals are segregated or slaughtered. suggested that the incidence of infection has to be reduced to about 4% of the bovine population before a slaughter-based eradication programme is likely to be feasible. As eradication progresses, the proportion of false positives from the cross reaction between S19 and virulent strains reaches a point at which it is more cost effective to desist with vaccination. Laterin the eradication process, monitoring at a herd level (e.g., using bulk milk) or in markets or at slaughter is more cost effective than individual animal testing.

Phase 4: Freedom

Criteria that have to be met to allow a region to be officially brucellosis free include that the condition is notifiable, that reactors are slaughtered, that vaccination is not used, and that the national/regional brucellosis infection rate has not exceeded 0.2% for at least 2 years. These criteria are reflected in current European Union regulation.

Conclusion

Brucellosis is a chronic infectious disease caused by small, non-motile, non-sporing, gram-negative, facultative intracellular coccobaccilli of the genus *Brucella.* The Rose Bengal test is used as a screening test and positive results are confirmed by the serum agglutination Tests. It is the most important lab spread disease. It is zoonoitic in nature. As brucellosis poses health threats to humans, and morbidity in untreated diseases is substantial, thus early consideration and diagnosis of brucellosis is important. As brucellosis is often misdiagnosed or overlooked, physicians in both endemic and nonendemic areas must be aware in their diagnosis of febrile diseases.

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