



Human brucellosis: A silent but dreadful disease

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Abstract: Brucellosis is a major bacterial zoonosis of human beings transmitted usually by direct contact with infected animals, their secretions and consuming milk or milk products. The disease occurs worldwide in animals as well as humans. Brucellosis in human beings is a multisystem disease with severe clinical manifestations in patients depending upon the site of infection and organ involved. Human brucellosis affects all age groups and considered as one of the most common laboratory acquired infections. The disease often remains under-diagnosed and under-reported due to poor seroprevalence studies which further pose difficulty in the eradication program. By following suitable brucellosis eradication strategies in animals, human brucellosis can correspondingly be controlled. The present article discusses about different aspects of human brucellosis and its control.

Keywords: *Brucella*, Control, Human beings, Zoonosis

Received: 15 August 2014 / Accepted: 01 September 2014 / Published Online: 25 September 2014

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Brucellosis is one of the most important bacterial zoonotic diseases affecting animals and humans. The disease is caused by various species of genus *Brucella*, a Gram negative, facultative intracellular, coccobacillary organism that is non-flagellar, non-capsulated and non-spore forming. Among the various species of *Brucella*, *B. abortus* (infecting bovines), *B. melitensis* (infecting goat and sheep), *B. suis* (infecting pigs) and *B. canis* (infecting dogs) also cause disease in human beings named as Human Brucellosis (Sprague et al. 2012). *B. melitensis* is most pathogenic in humans, followed by *B. abortus*, *B. suis* and *B. canis* in descending order, but *B. ceti* (infecting marine animals: whales, dolphins), *B. inopinata* and *innopinata* like species have also been reported to cause infection in humans, however, their pathogenicity is currently under investigation (Al Dahouk et al. 2013). The disease has many synonyms as Mediterranean fever, Malta fever, Gibraltar fever and Cyprus fever, on the basis of geographical regions in which disease occurs, undulant fever on the basis of characteristic undulating fever, or typho-

-homalarial fever and intermittent typhoid, on the basis of resemblance to malaria and typhoid (Mantur et al. 2007). It is a life threatening multisystem disease that may present with a broad spectrum of clinical manifestations.

Epidemiology

Human brucellosis is reported worldwide with geographical distribution changing constantly due to emergence or re-emergence of new foci. The disease is endemic in Mediterranean countries of Europe and Africa, Middle East, India, central Asia, Mexico, Central and South America. The disease has not been reported in some countries (Australia, Canada, Cyprus, Denmark, Finland, Netherland, New Zealand, Norway, Sweden and United Kingdom) where bovine brucellosis has been eradicated. More than 500,000 cases of human brucellosis are being reported every year (Pappas et al. 2006), however, the number of undetected cases believed to be much more which can be attributed because of non-specific clinical signs and symptoms, lack of awareness about disease in endemic areas, poor surveillance and laboratory facilities. The disease affects all age groups with well-characterized occupational risk in shepherds, farmers, abattoir workers, dairy industry professionals, veterinarians and personnel working in microbiology laboratories. Brucellosis has been recognized as one of the common laboratory-

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transmitted infections and reported to occur in clinical, research and production laboratories (Noviello et al. 2004).

Indian scenario

High prevalence of bovine brucellosis in Indian bovine population is a major risk factor for human being as humans and animals share the same socio-economic fabric in India. Veterinarian, animal attendants, animal vaccinators, farmers, slaughter house workers are at a higher risk of infection due to close contact to animal fluid/products. The seroprevalence of Bovine brucellosis was found to be 1.8% in buffaloes and 1.9% in cattle in the Indian subcontinent in 19 Indian states (Isloor et al. 1998) whereas Upadhyay and coworkers (2000) reported the overall seroprevalence as 7.25% in cattle and buffaloes from Uttar Pradesh. High seroprevalence (11.23%) was reported from Punjab which varies from 0% to 24.3% in various rural areas (Dhand et al. 2005). Mudliar and coworkers (2003) showed that 5.31% of dairy animal handlers are positive for brucellosis. Seroprevalence of Human brucellosis indicated wide variations: 26.6% in Ludhiana (Punjab) (Yohannes et al. 2011), 19.83% in Maharashtra (Aher et al. 2011), 11.51% in Andhra Pradesh (Mrunalini et al. 2011) and 0.8% in Kashmir (Kadri et al. 2000). More states need to be involved in routine serosurveillance of Human Brucellosis to get a clear picture of the situation.

Transmission

Infection in humans is usually transmitted from the animal hosts by direct contact with the secretions of infected animal or indirectly by consuming the contaminated foods (unpasteurized milk, milk products and undercooked meat products). The infection may also occur through abraded skin, conjunctiva or inhalation of airborne particles. Direct person to person spread of the disease is extremely rare, however, sexual transmission has also been reported (Kato et al. 2007). Breast feeding mothers may transmit the infection to their infants (Arroyo Carrera et al. 2006).

Clinical signs

The disease may occur in acute, sub-acute or chronic forms with incubation period ranging from 1-3 weeks to several months. Infections associated with *B. melitensis*, being more pathogenic, are usually acute (less than 2 months) and infections with other species may be sub-acute (2-12 months) or chronic (more than 12 months) in nature. Specific clinical signs and symptoms of brucellosis in humans are not reported, however, patients primarily may show undulating fever of unknown origin with multiple signs and symptoms in later stages as insomnia, anorexia, joint pain, lower back pain, cephalgia, fatigue, malaise, myalgia, night sweats, weight loss and several other symptoms (Mantur et al. 2007). Abortions mostly in first and second trimester of pregnancy have also been reported in pregnant women (Khan et al. 2001).

Human brucellosis presents a wide spectrum of clinical manifestations depending on the site of infection and the organ involved. Sauret and Vilissova (2002)

reviewed the clinical manifestations of human brucellosis as anaemia, deep vein thrombosis, endocarditis, hepatomegaly, leukocytoclastic vasculitis, leukopenia, liver abscess, lymphadenopathy, meningitis, nephritis, optic neuritis, pancytopenia, papilledema, splenic abscess, splenomegaly, spondylitis, thrombocytopenia and uveitis.

DIAGNOSIS

Due to wide spectrum of clinical manifestations and no specific clinical signs, early diagnosis of human brucellosis is difficult. So, the laboratory diagnosis along with history and clinical signs is performed.

History and clinical signs

History of the patient's exposure to animals and clinical signs as undulant fever is helpful in early diagnosis of human brucellosis. As there are no specific signs, the disease usually remains undiagnosed or misdiagnosed by the physicians, in such situations; laboratory diagnosis becomes important.

Bacterial isolation and identification

Isolation and identification of infectious agent is still considered gold standard for the definitive diagnosis. *Brucella* spp. can be isolated from blood, bone marrow, tissues (in focal complications) and body fluids, however, the isolation is dependent on stage of the disease, type of samples taken and culture methods used. Isolation from bone marrow is considered to be more sensitive at any stage of disease than blood but should be restricted to specific cases only because of painful procedure of sample collection (Gotuzzo et al. 1986). Blood agar, chocolate agar, trypticase soya agar and serum dextrose agar are used for culturing and some strains may require bovine or equine serum for growth. Selective media (Ferrell's medium) can also be used for contaminated tissue samples. The inoculated plates are incubated at 35-37°C in 5-10% CO₂ for 3-5 days, however, plates are observed up to four weeks before declaring the sample negative because of its fastidious nature. Colonies of smooth strains of *Brucellae* appear as raised, convex, translucent, non-pigmented and non-hemolytic with size of 0.5-1.0 mm on agar plates which are identified microscopically (Gram negative coccobacilli resembling fine sand) and biochemically (Oxidase positive, Urease positive etc.) further (Al Dahouk et al. 2003a). Morphology of *B. canis* colonies is rough and appears dull, yellowish and opaque. Culture methods have limitations of slow growth and low sensitivity.

"Remember the samples should be collected with appropriate precautions and handled in biosafety level 2 while the cultures must be handled in biosafety level 3 laboratory"

Serological diagnosis

Serological tests are indirect measures of infection by detecting high titers of specific antibodies. Antigen (Ag) used for serological diagnosis of *Brucella* infections is usually made of whole cell extracts containing

smooth lipopolysaccharides (S-LPS) as the major fraction. However, due to cross-reactivity with other bacteria as *Yersinia enterocolitica* O:9, *Salmonella urbana* group N, *Vibrio cholerae*, *Escheichia coli* O:157 etc, specificity of these assays is low (Al Dahouk et al. 2003b). Infections with *B. canis* cannot be detected by the S-LPS based assays as S-LPS Ag is not shared because of rough colony characters (Lucero et al. 2005). These tests are fast, having less risk of laboratory acquired infections and more sensitive than culturing method. Various serological tests used for diagnosis of human brucellosis are:

Serum agglutination test (SAT)

Brucellosis was first diagnosed serologically by applying simple tube agglutination test (Wright and Smith 1897). Subsequently, various modifications to the tube agglutination test were made to improve the test accuracy as slide, plate and card agglutination tests. This test is extensively used in endemic regions for rapid screening of population (Rose Bengal Test). Coombs' test is another agglutination test usually performed in chronic and relapsed cases where SAT is either negative or inconclusive. BrucellaCapt is a single step immunocapture agglutination test used for the detection of total antibodies against *Brucella* and suggested as a possible substitute for Coombs' test. This test can be used at any stage of the disease and the titres decrease slowly in relapsed cases while titres decrease rapidly in successful antibiotic treatment cases (Casanova et al. 2009).

Enzyme linked immunosorbent assays (ELISA)

These are indirect primary binding assays and detect the antibodies present in test sample by using antiglobins/bacterial cell receptors labeled with isotopes, fluorochromes or enzymes as detecting molecules. ELISA has been found to be an acceptable alternative to blood culture for the diagnosis of brucellosis (Shamahy and Wright 1998).

Lateral flow assay

Colored beads conjugated with a reagent are used in these assays to detect the antibodies bound to an immobilized antigen on a cellulose membrane. Appearance of a visible colored line is indicator of positive reaction (Kim et al. 2007). These assays are easy to perform and suitable for rapid field testing particularly in those endemic areas where laboratory facilities are poor. Various other assays used for serodiagnosis of brucellosis as fluorescence polarization assay (FPA), Fluorescence immunoassay using a capture and elution technique, chemi-luminescence assays etc. have been reviewed by Nielsen and Yu (2010).

Molecular diagnosis

Polymerase chain reaction (PCR) and various assays based on PCR (Real-time PCR, genus-specific PCR, species-specific PCR, Bruce-ladder PCR etc.) are used to detect *Brucella* DNA from clinical samples and cult-

ures. These assays are fast, can be performed on any clinical specimen, small quantity of sample required, more sensitive than blood cultures and more specific than serological tests. However, *Brucella* DNA remains detectable in majority of patients throughout the treatment period and even after treatment, indicative of relapse or chronic form of the disease. This might be because of survival and persistence of bacteria in macrophages and quantitative real-time PCR could overcome this problem of conventional PCR assays (Queipo-Ortuno et al. 2008).

Treatment

Brucellosis is treatable in humans by following appropriate antimicrobial therapy for a definite period. Many antimicrobial agents are effective against human brucellosis viz. doxycycline, rifampicin, gentamicin, trimethoprim-sulfamethoxazole, streptomycin, tetracycline, Quinolones etc. but usually a combination of antibiotics has been recommended to prevent the treatment failure and high rates of relapse. Treatment regimens recommended for adults are:

- a) Doxycycline Rifampicin Regimen: Doxycycline (100 mg p.o. bid x 6 weeks) + Rifampicin (600-900 mg/d p.o. x 6 weeks) WHO, 1986, or
- b) Gentamicin Rifampicin Regimen: Gentamicin (2 mg/kg q 8 hr i/v or i/m x 7 days) + Rifampicin (600-900 mg/d p.o. x 6 weeks), Solera et al. 1997 or
- c) Ciprofloxacin Rifampicin Regimen: Ciprofloxacin 1 g p.o. qd x 30 d) + Rifampicin (600 mg p.o. qd x 30 d), Agalar et al. 1999

Childhood brucellosis can be successfully treated with doxycycline @ 4 mg/kg/day + rifampicin @ 10 mg/kg/day orally for six weeks (Mantur et al. 2004). Rifampicin with or without a combination of cotrimoxazole has been proved safe to treat brucellosis during pregnancy (Ozbay and Inanmis 2006). However, the choice of combination and duration of antimicrobial therapy should be based on the location of disease and the underlying conditions. It is advised to follow-up the cases to assess the response to therapy using appropriate serological tests.

Control

Control of human brucellosis mainly depends on the eradication of disease in animals, hygienic measures to prevent the spread of infection and the effective heating of dairy and meat products. Once the disease has occurred in humans, early stage diagnosis and appropriate antimicrobial therapy is the only action to prevent serious complications in patient. For an effective control of brucellosis in animals, the following steps are essential:

- i) Effective surveillance to identify the infection,

- ii) Prevention of transmission to healthy animals,
- iii) Eradication of reservoir to eliminate the source of infection and
- iv) Vaccination of female calves and goats.

Live attenuated vaccines containing *B. abortus* strain 19 are most commonly used in bovines. The vaccine is used only in female calves aged 4-9 months. Strain RB 51 has also been approved as vaccine strain in USA. Rev-1 strain of *B. melitensis* is used as vaccine for control of brucellosis in sheep and goats; however, this strain shows considerable degree of virulence and may cause abortions in pregnant animals. Vaccines in humans have not been used because of limited efficacy and serious medical reactions, although attempts have been made in the former Soviet Union and China (Seleem et al. 2010).

In brucellosis free countries, movement of infected animals must be prohibited and there must be strict regulations of import of animals and their products only from certified brucellosis free countries.

Future perspectives

The disease largely remains undiagnosed/ misdiagnosed and underreported. For the success of eradication program, there must be effective surveillance system in animals, proper laboratory facilities and standardized tests for diagnosis, mass vaccination of animals, and reimbursement strategy for farmers for elimination of infected animals and extensive cooperation and exchange of information between veterinary and medical services. Urgency of the time is to strictly implement the eradication strategies for brucellosis in animals in the affected regions.

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