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Review Article

REVIEW OF CLINICAL AND LABORATORY FEATURES OF HUMAN BRUCELLOSIS

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Abstract

Infection with *Brucella* spp. continues to pose a human health risk globally despite strides in eradicating the disease from domestic animals. Brucellosis has been an emerging disease since the discovery of *Brucella melitensis* by Sir David Bruce in 1887. Although many countries have eradicated *B. abortus* from cattle, in some areas *B. melitensis* and *B. suis* have emerged as causes of this infection in cattle, leading to human infections. Currently *B. melitensis* remains the principal cause of human brucellosis worldwide including India. The recent isolation of distinct strains of *Brucella* from marine mammals as well as humans is an indicator of an emerging zoonotic disease. Brucellosis in endemic and non-endemic regions remains a diagnostic puzzle due to misleading non-specific manifestations and increasing unusual presentations. Fewer than 10% of human cases of brucellosis may be clinically recognized and treated or reported. Routine serological surveillance is not practiced even in *Brucella* - endemic countries and we suggest that this should be a part of laboratory testing coupled with a high index of clinical suspicion to improve the level of case detection. The screening of family members of index cases of acute brucellosis in an endemic area should be undertaken to pick up additional unrecognised cases. Rapid and reliable, sensitive and specific, easy to perform and automated detection systems for *Brucella* spp. are urgently needed to allow early diagnosis and adequate antibiotic therapy in time to decrease morbidity / mortality. The history of travel to endemic countries along with exposure to animals and exotic foods are usually critical to making the clinical diagnosis. Laboratory testing is indispensable for diagnosis. Therefore alertness of clinician and close collaboration with microbiologist are essential even in endemic areas to correctly diagnose and treat this protean human infection. Existing treatment options, largely based on experience gained > 30 years ago, are adequate but not optimal. In our experience, an initial combination therapy with a three drug-regimen followed by a two-drug regimen for at least six weeks and a combination of two drugs with a minimum of six weeks seems warranted to improve outcome in children and adult patients respectively with laboratory monitoring. A safe and effective vaccine in humans is not yet available. Prevention is dependent upon the control of the disease in animal hosts, effective heat treatment of dairy produce and hygienic precautions to prevent occupational exposure. This review compiles the experiences and diagnostic and treatment paradigms currently employed in fighting this disease.

Key words: *Brucella spp, combined and prolonged therapy, epidemiology, protean manifestations, serological surveillance*

Historical Perspective

Brucellosis is a zoonosis transmitted to humans from infected animals. 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. Much later in the 19th century, the disease was found to affect British armed forces and the local population of Malta. J. A. Marston, an assistant surgeon of the British Medical Department working in the Mediterranean in 1861, first described the symptoms of brucellosis in himself as" gastric remittent fever."[1] Brucellosis has many synonyms derived from the geographical regions in which disease occurs e.g., Mediterranean fever, Malta fever, Gibraltar fever, Cyprus fever; from the remittent character of the fever e.g., undulant fever; or from its resemblance to malaria and typhoid e.g., typhomalarial fever, intermittent typhoid. The cause of this disease was obscure until 1887 when Sir David Bruce - a Scottish physician reported numerous small coccal organisms in stained sections of spleen from a fatally infected soldier and isolated and identified organism in culture from spleen tissue of four other British soldiers stationed at Malta.[2] This organism, which he designated *Micrococcus melitensis*, produced a remittent fever in inoculated monkeys. One animal died from the infection and the organism was recovered in pure culture from the liver and spleen. The organism derived its species name from Melita (honey), the Roman name for the Isle of Malta. Hughes ML, in a monograph in 1897, portrayed the findings in people in greater detail, emphasizing "undulant fever" and suggested the name undulant fever.[3] Wright and Smith in 1897 detected antibodies to *M. melitensis* in human and animal sera through agglutination test, which unravelled the zoonotic potential of the disease.[4] Later, Zammit an young Maltese physician working with Mediterranean Fever Commission in 1905 confirmed it by isolating the organism

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from the milk and urine of goats.[5] Thus he concluded that the goat was the reservoir of *M. melitensis* and the consumption of the raw milk and cheese infects man.

In the same year that Hughes monograph appeared, Bang in Denmark isolated a gram-negative rod from cattle, which had aborted. The third member of the group, which also is bacillary in shape was recovered from the foetus of aborted swine by Traum in 1914 in the United States of America and implicated as an agent of brucellosis in man by Huddleson in 1943. In 1918, Alice Evans an American bacteriologist published reports which contained convincing evidence that *M. melitensis* from goats and a gram-negative rod from cows could not be differentiated morphologically or by their cultural and biochemical reactions but there were antigenic differences which could be shown by agglutination absorption test. She also showed in 1920 that *M. melitensis* was also a bacillus. She showed that *M. melitensis*, isolates of cows and pigs belonged to one genus. Meyer and Shaw[6] further confirmed Evan's observations and suggested the generic name *Brucella* in honour of Sir David Bruce. The possible pathogenicity of *B. abortus* to man was suggested by Evan in 1918 and confirmed by others.

In 1956, Buddle and Boyce discovered *B. ovis*, the cause of epididymitis in rams. In 1957, Stoenner and Lackman isolated *B. neotomae* from desert wood rat in Utah in USA. In 1968, Carmicheal and Bruner discovered *B. canis* as the cause of an epidemic of abortions in beagles. Human infections due to *B. canis* have been reported.[7] Two new *Brucella* species, provisionally called *B. pinnipediae* and *B.cetaceae*,have been isolated from marine hosts within the past few years.[8,9] There are three reports in the literature of humans infected with marine mammal strains of *Brucella*; one infection occurred in a research laboratory worker after occupational exposure,[10] and the other two were community- acquired infections[11,12] including the recent report in a patient of New Zealand with spinal osteomyelitis.

Taxonomy

The taxonomy of *Brucella* species is still unclear and unresolved. Based on 16S rRNA gene sequences, *Brucellae* are categorised as α-2 proteobacteria and have close phylogenetic relationships with *Agrobacterium*, *Rickettsia*, *Rhizobium* and *Rhodobacter*.[13] *Brucellae* have been classified according to differences in pathogenicity and host preference, into six species: *B. melitensis, B. abortus, B. suis, B. ovis, B. canis* and *B. neotomae.* In fact Verger and colleagues used DNA-DNA hybridization studies to investigate 51 *Brucella* strains of all species and found them to be identical.[14] With these results, they proposed that all species should be considered as biovars of *B. melitensis.* However, because of the differences in the animal reservoirs and in the severity of clinical disease associated with the different species, this proposal has not been widely accepted. Table 1 summarizes the taxonomic characteristics of *Brucella* species.[15]

Causative Agents, Sources and Modes of Transmission

Brucellae are facultative intracellular, gram-negative, partially acidfast coccobacilli that lack capsules, flagellae, endospores or native plasmids. The bacterium is of 0.5-0.7μ in diameter and 0.6-1.5μ in length. They are oxidase, catalase and urease positive. *Brucella* species considered as important agents of human disease are *B. melitensis, B. abortus and B. suis*.[16] The transmission of *Brucella* infection and its prevalence in a region depends upon several factors like food habits, methods of processing milk and milk products, social customs, husbandry practices, climatic conditions, socio-economic status and environment hygiene. Environmental sanitation is particularly important in the context of air borne transmission.[17] Brucellosis is almost invariably transmitted to man from infected domestic animals. However, the possibility of human to human transmission of *Brucella* infection has also been reported in the literature.[18-23] Human brucellosis was once thought to be predominantly transmitted through animal contact. However, it is now being realized increasingly that animal products such as milk and meat products also play important role in the disease transmission. Dairy products prepared from unpasteurized milk such as soft cheese, yoghurts and ice-creams may contain high concentration of the bacteria and consumption of these is an important cause of brucellosis.[24] It is the commonest mode of transmission in case of *B. melitensis* and *B. abortus* infections in general population. Camel milk is also considered to be the important source of infection in Middle East countries and Mongolia. Bacterial load in animal muscle tissues is low, but consumption of undercooked traditional delicacies such as liver has been implicated in human infection.[25] Other means of infection include skin abrasions[26] or inhalation of airborne animal manure particles.[27] In addition, laboratory acquired *Brucella* infection due to accidental ingestion, inhalation and mucosal or skin contact is a major health hazard for the laboratory workers handling the cultures of the virulent or attenuated *Brucella* strains. The disease has been recognized as one of the common laboratory- transmitted infections and has been reported to occur in clinical, research, and production laboratories.[10,28-31] Increased business and leisure travel to endemic countries have led to diagnostic challenge in areas where brucellosis is uncommon.

Antigenic Components

A substantial number of antigenic components of *Brucella* have been characterized. However, the antigen that dominates the antibody response is the lipopolysaccharide (LPS). LPS of non-smooth strains (R-LPS) is similar to LPS of smooth strains (S-LPS) except that the O-chain is either absent or reduced to a few residues. Strong cross-reactions in both the agglutination and complement fixation tests have been reported between smooth species of *Brucella* and *Yersinia enterocolitica* O:9, *Escherichia hermanni, Escherichia coli* O:157, *Salmonella* O:30, *Stenotrophomonas maltophila* and *Vibrio cholerae* O:1.[32] These have been

Table 1: Taxonomic characteristics of *Brucella* species

Species	Biotypes	Host reservoir	Described by	Species identification
B. melitensis	1-3	Goats, sheep, camels	Bruce, 1887	Fuchsin, positive; thionin, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 24 hr; CO_2 growth, negative; Tiblisi phage lysis, negative; Weybridge phage lysis, negative
B. abortus	1-6,9	Cows, camels, Yaks, buffalo	Bang, 1897	Fuchsin, positive (except biotype 2); thionin,negative (biotypes 1, 2, and 4); safranin inhibition, negative; H_2S production, positive (except biotype 5); urease, positive in 24 hr; CO_2 growth, positive (biotypes 1-4); Tiblisi phage lysis, positive; Weybridge phage lysis, positive
B. suis	1-5	Pigs (biotypes 1-3), wild hares biotype 2), caribou (biotype 4), reindeer (biotype 4), wild rodents (biotype 5)	Traum, 1914	Fuchsin, negative (except biotype 3); thionin, positive; safranin inhibition, positive; H_2S production, positive (biotype1); urease, positive in 15 min; CO_2 growth, negative; Tiblisi phage lysis, negative; Weybridge phage lysis, positive
B. canis	--	Canines	Carmichael and Bruner, 1968	Fuchsin, positive or negative; thionin, positive; safranin inhibition, negative; H_2S production, negative; urease, positive in 15 min; CO_2 growth, negative; Tiblisi phage lysis, negative; Weybridge phage lysis, negative
B. ovis	--	Sheep	Van Drimmelen, 1953	Fuchsin, negative for some strains; safranin inhibition, negative; H_2S production, negative; urease, negative; CO_2 growth, positive; Tiblisi phage lysis, negative; Weybridge phage lysis, negative
B. neotomae	--	Rodents	Stoenner and Lackman, 1957	Fuchsin, negative; safranin inhibition, negative; H_2S production, positive; urease, positive in 15 min; CO_2 growth, negative; Tiblisi phage lysis, positive or negative; Weybridge phage lysis, positive
B. pinnipediae and *B. cataceae* (provisional)	--	Mink whales, dolphins, porpoises (*pinnipediae*), seals (*cetaceae*)	Ewalt and Ross, 1994	Fuchsin, positive; thionin, positive; safranin inhibition, negative; H_2S production, negative; urease, positive; CO_2 growth, negative for *pinnipediae* and positive for *cetaceae*; Tiblisi phage lysis, negative; Weybridge phage lysis, positive for *pinnipediae* and negative for *cetaceae*

attributed to similarities on the O-specific side chains of the lipopolysaccharide molecule of the organisms. Numerous outer and inner membrane, cytoplasmic and periplasmic protein antigens have also been characterized. Some are recognized by the immune system during infection and are potentially useful in diagnostic tests.[33] Omp25 is an outer membrane structural protein that is highly conserved in all *Brucellae*. It is associated with both lipopolysaccharide and peptidoglycan. Recently, ribosomal proteins have emerged as immunologically important components since they confer protection against challenge with *Brucella* on account of both antibody and cell mediated responses.[34] One such example is L7/L12. This elicits delayed hypersensitivity response as component of brucellins[35] and as fusion proteins, which has been shown to stimulate protective response.[36] Hence this appears to have potential as candidate vaccine component.

Genome

In the initial years of this decade, the complete genomic sequence of *B. melitensis, B. abortus and B. suis* has been achieved.[37-39] The average size of the genome is 2.37 x 10^9 daltons, with a DNA G + C content of 58-59mol%.[40] All types show > 95% homology in DNA-DNA pairing studies, justifying the nomination of *Brucella* as a monospecific genus. Restriction fragment patterns produced by infrequently cutting endonucleases support the differentiation of the nomen species.[41] Restriction endonuclease analysis has generally been unsuccessful for strain differentiation, but polymerase chain amplification of selected sequences followed by restriction analysis has provided evidence of polymorphism in a number of genes including omp 2, dnaK, htr and ery (the erythrulose-1-phosphate dehydrogenase gene).[42-44] The omp 2 gene is believed to determine dye sensitivity, one of the traditional typing methods for biotype differentiation. Its polymorphism and the capacity for post-translational modification of its product may explain the tendency for variation in dye sensitivity patterns even within species and have been used as the basis for a genetic classification of *Brucella*.[45-46] A 7.2 kbp deletion in the ery gene in *B. abortus* strain 19[44] may explain the erythritol sensitivity of this strain which is a major factor in its attenuation. The genome of *Brucella* contains two circular chromosomes of 2.1 and 1.5 Mb, respectively. Both replicons encode essential metabolic and replicative functions and therefore are chromosomes, not plasmids.[47-48] Natural plasmids have not been detected in *Brucella*, although transformation has been effected by wide host range plasmids following conjugative transfer or electroporation.[49]

Virulence Factors, Pathogenic Mechanisms and Immune response

The pathogenicity in human brucellosis is related to various factors. The S-LPS is a major determinant of virulence and dominates the antibody response. It is the main component responsible for conferring incomplete and short-term protection against infection in passive transfer experiments with monoclonal and polyclonal antibodies.[50] The elimination of virulent *Brucellae* depends upon activated macrophages and hence requires development of Th1 type cell-mediated immunity. *Brucella* LPS is a relatively poor inducer of gamma-interferon and tumour necrosis factor-α, both of which are essential for the elimination of the organism.[51,52] Unusually, it is an effective inducer of interleukin 12, which stimulates Th1 type response and is closely correlated with gamma interferon production. Other important virulence factors include: production of inhibitors of phagolysosome fusion such as adenine and guanine monophosphate levels;[53] outer membrane protein 25 which has been identified as the down regulator of TNF alpha[54] especially in an early stage of infection. This leads to impaired activation and cytotoxic function of natural killer cells. The phenotypic difference and host preference of *Brucella* spp. can be attributed to various specific outer membrane protein markers.[55] Survival within macrophages is also associated with the synthesis of stress induced proteins of different molecular weight, notably 24 kDa which induces acid environment of pH< 4. This acid environment is also responsible for limiting antibiotic action and explains the discrepancy between *in vitro* studies and *in vivo* events.[56] Recently urease enzyme has been identified as an important determinant of virulence.[57] Urease has the role to protect *Brucellae* in their passage through the stomach when acquired by the oral route, which is the major way of infection in human brucellosis. All these factors probably play a substantial role in the intracellular survival of at least 15 to 30% of *Brucellae* ingested and these *Brucellae* start replicating in the endoplasmic reticulum. After entering the human body and being taken up by local tissue lymphocytes, *Brucellae* are transferred through regional lymphnodes into the circulation and are subsequently seeded throughout the body, with tropism for the reticuloendothelial system.

Epidemiology

The epidemiology of brucellosis is complex and it changes from time to time. Wide host range and resistance of *Brucellae* to environment and host immune system facilitate its survival in the populations.

Global scenario

Worldwide, brucellosis remains a major source of disease in humans and domesticated animals. The disease is endemic especially in countries of the Mediterranean basin, the Arabian gulf, the Indian subcontinent and parts of Mexico and Central and South America. Human brucellosis is found to have significant presence in rural/nomadic communities where people live in close association with animals. Worldwide, reported incidence of human brucellosis in endemic disease areas varies widely, from <0.01 to >200 per 100,000 population.[58] The true incidence of human brucellosis however, is unknown for most countries including India. It has been estimated that the true incidence may be 25 times higher than the reported incidence due to misdiagnosis and underreporting. It has been shown that the incidence of human brucellosis is significantly high where ovine/caprine brucellosis caused by *B. melitensis* is endemic.[59] Recent re-emergence in Malta and Oman indicates the difficulty of eradicating this infection.[60] Sheep and goats and their products remain the main source of infection, but *B. melitensis* in cattle has emerged as an important problem in some southern European countries, Israel, Kuwait and Saudi Arabia. *B. melitensis* infection is particularly problematic because *B. abortus* vaccines do not protect effectively against *B. melitensis* infection; the *B. melitensis* Rev.1 vaccine has not been fully evaluated for use in cattle. Despite vaccine campaigns with Rev.1 strain, *B. melitensis* remains the principal cause of human brucellosis worldwide. In some South American countries, particularly Brazil and Colombia *B. suis* biovar 1 has become established in cattle.[61] In some areas, cattle are now more important than pigs as a source of human infection. Screening of household

members of an index case is important epidemiological step since this picks up additional unrecognised cases.[62,63] This must be taken into account by the family clinicians caring for these patients, so that timely diagnosis and provision of therapy occur, resulting in lower morbidity. The recent isolation of distinctive strains of *Brucella* from marine mammals[8,9] as well as humans[10-12] has extended the ecological range of human brucellosis. Because new strains may emerge and existing types adapt to changing social and agricultural practices, the picture remains incomplete.

It is a well-characterized occupational disease in shepherds, abattoir workers, veterinarians, dairy industry professionals and personnel in microbiologic laboratories. Males are affected more commonly than females,[63] which may be due to risk of occupational exposure. Human brucellosis affects all age groups.

Indian scenario

The occurrence of brucellosis in India was first established early in the previous century and since then has been reported from almost all states.[64,65] Several publications indicate that human brucellosis can be a fairly common disease in India. Mathur reported 8.5% seroprevalence of brucellosis among dairy personnel in contact with infected animals with the isolation of *Brucella* strains from seven cases of human brucellosis.[66] As many as 4.2% aborted women were seropositive for the disease.[67] In Gujarat, 8.5% prevalence of *Brucella* agglutinins was recorded in human cases.[68] In Haryana, 34% prevalence of human brucellosis was recorded among veterinarians, attendants and compounders in contact with animals.[69] The study conducted by Thakur *et al.*[70] revealed a prevalence rate of 4.97% in samples obtained from persons exposed to animals with the markedly higher prevalence of 17.39% among field veterinarians. In a study conducted by Hemashettar *et al.*[71] 24(8.2%) veterinary workers showed *Brucella* specific antibodies in significant titres. High seroprevalence rate has been also noted in specific risk groups such as abattoir workers.[72,73] These observations support the occupational risk factors for brucellosis.

Since uncharacterised fever is the only manifestation in a large number of patients some workers screen pyrexia of unknown origin (PUO) cases for evidence of brucellosis. Handa and coworkers identified four (3.3%) cases with acute brucellosis in a group of 121 patients with PUO.[74] Sen and co-workers identified 28 (6.8%) seropositive cases in a group of 414 patients with PUO and Kadri and co-workers identified 28 (0.8%) seropositive cases in a group of 3,532 patients with PUO.[75,76]

A prevalence of 3% was observed among patients attending Karnataka Medical College, Hubli.[77] A study by Mantur and colleagues[78] reported 93 children with brucellosis identified from 5726 children in Bijapur during a period of 13 years. The seroprevalence was 1.6% by standard tube agglutination test (≥ 1:160) and the diagnosis was confirmed in 43 of these paediatric patients by the isolation of *B. melitensis*. A recent publication by Mantur and colleagues[63] reported 495 adults with brucellosis with the prevalence of 1.8% who were identified by testing blood samples from 26,948 adults in Bijapur during a period of 16 years from 1988 to 2004 and isolated *B. melitensis* from 191 cases. Subsequent continuation of the study after publications in Bijapur till 8th April 2006, diagnosed additional 111 cases of brucellosis from testing 6765 blood samples (Mantur BG, unpublished work). A separate study by Mantur and colleagues identified 11(0.62%) patients by SAT (≥ 1:160) and isolated two *Brucella* strains, one from blood and the other from testicular fluid by testing blood samples from 1750 patients seen at Belgaum Institute of Medical Sciences in Belgaum during the period June 2006 to January 2007 (Mantur BG and colleagues, unpublished work).

Spectrum of Disease

Brucellosis is a systemic disease that can involve any organ or system of the body. Four species are responsible for most human cases: *B. melitensis* (found in sheep and goats), *B. abortus* (found in cattle), *B. suis* (found in swine) and *B. canis* (found in dogs). Disease from marine species has also emerged.[10-12] *B. melitensis* remains the principal cause of human brucellosis worldwide. A recent study did not report any clinical difference between cases caused by *B. melitensis* and *B. abortus*.[79] Sufficient data on virulence and clinical presentation of biotypes of *B. melitensis* are lacking, although separate biotypes that predominate in various regions such as type 1 in India[63,78] and Spain,[80] type 2 in northwestern Greece and type 3 in Turkey[81] may account for variations in clinical presentation. The infective dose of *Brucella*, especially that of *B. melitensis* is very low (10 organisms). The incubation period is usually between seven days and three months, although as long as 10 months have been reported.[82]

Human brucellosis is known for presenting with protean manifestations[63,78] (Table 2). However, the most common presenting symptom is fever. Some authors[83] consider malodorous perspiration as almost pathognomonic. Pityriasis alba was found to be the consistent physical finding, with fever in the majority of patients suffering from childhood brucellosis.[78] Human brucellosis usually manifests as an acute (< 2 months) or subacute (2-12 months) febrile illness, which may persist and progress to a chronically (> 1 year) incapacitating disease with severe complications. Some authors[83] have considered this classical categorization to be of limited clinical interest. Persons infected with *Brucella* spp. usually have signs and symptoms consistent with an influenza like or septicaemic illness, often with insidious onset. The symptoms and clinical signs most commonly reported are fever, fatigue, malaise, chills, sweats, headaches, myalgia, arthralgia and weight loss.[63,78,84-87] Some cases have presented with only joint pain,[63,78] low back ache,[63] involuntary movements of limbs,[78] burning feet,[78] or ischemic

Table 2: Clinical findings in 740* patients infected with *B. melitensis*

Symptoms / signs	No. of patients (%)
Fever (> 37.5ºC)	576 (77.8)
Joint pain	156 (21)
Low backache	104 (14)
Night sweats	27 (3.6)
Cough, breathlessness, haemoptysis	25 (3.3)
Testicular pain, scrotal swelling, burning micturition	15 (2)
Pain in abdomen, nausea, vomiting, anorexia, jaundice	24 (3.2)
Headache	17 (2.2)
Fatigue	10 (1.3)
Papules**, mouth ulcers	11 (1.4)
Convulsions	2 (0.2)
Splenomegaly	128 (17.2)
Hepatomegaly	75 (10.1)
Hepatosplenomegaly	112 (15.1)
Lymphadenopathy	23 (3.1)
Jerky movements of limbs	1 (0.1)
Burning feet	1 (0.1)
Swollen hand	2 (0.2)
Weight loss	6 (0.8)

*Data of the institutions where cases were identified, **One case was also associated with subcutaneous nodules

heart attacks.[88] Medical literature[63,78] (Table 3) under reports brucellosis cases because of misleading clinical pictures.[89-91] Typically, no or few objective signs are apparent that specifically point to brucellosis. Enlargement of the liver, spleen and/or lymph nodes may occur as may other signs referable to almost any other organ system. These febrile patients may be referred to as patients with pyrexia of unknown origin or the symptoms and signs are confused with those of other diseases such as enteric fever, malaria, rheumatic fever, tuberculosis, cholecystitis, thrombophlebitis, fungal infection, autoimmune disease and tumours.[63,78,84,92-94] Thus to an unaware physician, the clinical diagnosis becomes a challenging one.

B. melitensis is associated with acute infection whereas the infections with other species are usually subacute and prolonged. The acute form of human brucellosis is characterised by an undulating fever, in addition to the signs and symptoms mentioned. The temperature remains normal during the early part of the day and rises during the evening. Lack of appropriate therapy during the acute phase may result in localization of bacteria in various tissues and lead to subacute or chronic disease that can have serious clinical manifestations.[16,91] Brucellosis in humans occurs in all age groups.[25,63,78,95] Brucellosis in children can be very common particularly with *B. melitensis*.[78,96,97] The clinical presentation, epidemiology, diagnosis and treatment outcome were similar to those seen in non human immunodeficiency virus (HIV) infected patients emphasizing a casual relationship of brucellosis and HIV infection in both endemic and non-endemic areas.[63,98-100]

Human brucellosis is known for complications. Complications can be very diverse depending on the specific site of infection.[101] Osteoarticular, genitourinary, gastrointestinal, nervous, cardiovascular, skin and mucous membranes and respiratory complications are observed. Bone and joint involvement is the most frequent complication of brucellosis and occurs in up to 40% of cases in some series.[102] Three distinct forms exist; peripheral arthritis, sacroilitis and spondylitis. Peripheral arthritis is the most common and is non-erosive, since it usually involves the knees, hips, ankles and wrists in the context of acute infection.[63,78,95,103] Epididymoorchitis[63,104] is the most frequent genitourinary complication in men and may be confused with testicular cancer or tuberculosis.[105,106] Brucellosis during pregnancy poses a substantial risk of spontaneous abortion or intrauterine transmission of infection to the infant.[107,108] As the largest organ of the reticuloendothelial system, the liver is probably involved in the majority of cases of brucellosis even though liver function tests are normal or values are usually only mildly abnormal. Invasion of central nervous system (CNS) occurs in about 5-7% of the cases of *B. melitensis* infection. Meningitis, encephalitis, meningoencephalitis, meningovascular disease, brain abscesses and demyelinating syndromes have all been reported.[63,78,95,109] *Brucellae* are rarely isolated from cerebrospinal fluid (CSF), but antibodies to *Brucella* species are present in the serum and CSF in the majority of cases.[63,78,95] *Brucella* endocarditis occurs in less than 2% of cases but accounts for the majority of *Brucella*-related deaths. Early recognition, adequate antibiotic treatment and the absence of signs of heart failure can guide the practitioner toward prolonged, conservative treatment.[110] Complications involving the skin, although rare, are reported in the literature.[63,78] Respiratory tract complications may be seen in abattoir workers and are thought to be caused by the inhalation of *Brucellae*.[111] A multinational review of cases by Pappas *et al.*[112] and a recent publication by Mantur and coworkers[63] indicate that the pulmonary involvement is not rare.

The reports of unusual manifestations with atypical lesions in brucellosis are on the rise due to availability of diagnostic facilities and awareness. Tsolia and colleagues[95] have noted unusual complications in two children; one developed acute left facial nerve palsy and the other thrombocytopenic purpura. We reported complications (arthritis excluded) in 9.7% (Table 4, most part published) of patients with unusual manifestations like chorea,[78] hydrocele,[63] Stevens-Johnson syndrome,[63] and urinary tract infection.[63]

Neurobrucellosis has been reported as an exceptional cause of transient ischemic attacks.[88] Very recently, we have identified in Belgaum, two patients with atypical lesions, one was with hemorrhagic epididymoorchitis and the other with

Table 3: Clinical diagnosis of 740* cases following initial examination

Principal / differential diagnosis	No. of cases (%)
Enteric fever	252 (34.05)
Malaria	120 (16.2)
Arthritis	70 (9.45)
Brucellosis	108 (14.6)
Pyrexia of unknown origin	62 (8.3)
Epididymo-orchitis, **bilateral hydrocele, urinary tract infection, pyonephrosis	16 (2.16)
Tuberculosis	8 (1.08)
Chronic liver disease, splenic abscess, acute cholecystitis	10 (1.35)
Endocarditis	11 (1.48)
Bronchitis, pnuemonia	9 (1.2)
Skin rashes, Stevens-Johnson syndrome, cellulitis	11 (1.48)
Meningitis	8 (1.08)
Human immunodeficiency virus infection	3 (0.4)
Encephalitis	5 (0.6)
Malaria, enteric fever, brucellosis***	2 (0.27)
Enteric fever, brucellosis***	41 (5.5)
Pulmonary tuberculosis, brucellosis***	1 (0.13)
Rheumatic arthritis, brucellosis***	1 (0.13)
Chorea	1 (0.13)
Peripheral neuritis	1 (0.13)

*Includes: (1) 30 cases from 1000 blood samples screened from KMC Hubli (1985-1987). (2) 699 cases from 39,439 blood samples screened in Bijapur (August 1988 to April 8, 2006, most part published).[63,78] (3) 11 cases from 1750 blood samples screened from Belgaum Institute of Medical Sciences, Belgaum (June 2006 to January 2007, unpublished). **One case was haemorrahagic; one case was also associated with cellulitis. ***Differential diagnosis.

Table 4: Complications of Brucellosis

Complication	No. of cases
Genitourinary (16)	
Epididymo-orchitis*	12
Hydrocele	02
Urinary tract infection	01
Pyonephrosis	01
Neurobrucellosis (15)	
Meningitis	08
Meningoencephalitis	05
Chorea	01
Peripheral neuritis	01
Endocarditis	11
Cutaneous/mucous membrane lesions**	11
Gastrointestinal tract (10)	
Chronic liver disease	08
Splenic abscess	01
Ac. Cholecystitis	01
Respiratory system (09)	
Pneumonia	04
Bronchitis	05
Total	**72**

*One case was haemorrhagic; one was associated with cellulitis. **Included a case of Stevens-Johnson syndrome and a case of cellulitis.

cellulitis. In conclusion, it should be noted that brucellosis may affect essentially any organ - a fact that reinforces the importance of including brucellosis in the differential diagnosis even if clinical features are not entirely compatible, especially in endemic areas.[83]

Laboratory Diagnosis

Clinician must develop a high degree of clinical suspicion based on epidemiological information. A thorough travel history as well as history of exposure to animals and exotic foods are usually critical to making the clinical diagnosis.[16] In all cases a blood sample should be collected from the patient and laboratory testing should be requested as the definite diagnosis of brucellosis is impossible without laboratory confirmation.[16] A proper and prompt diagnosis is important, as the treatment requires specific and prolonged antibiotics.[113]

Laboratory tools include isolation and identification of *Brucellae* from clinical samples, detection of antigen, demonstration of genome and demonstration of *Brucella* specific antibodies. Blood culture provides definite proof of brucellosis but may not provide a positive result for all patients even under ideal conditions.[80] *Brucellae* are relatively slow growing and the culture result may not become available for several days or weeks. In particular for patients with chronic disease, the sensitivity of culture can be low. Recently, higher rates of positive blood cultures (91% in acute brucellosis and 74% in chronic brucellosis) along with the rapid confirmation of clinical diagnosis have been reported by lysis centrifugation technique.[114] The modern automated blood culture systems have somewhat improved the speed of detection but are still too slow to make a rapid diagnosis.[115] Although bone marrow culture has been considered the gold standard for the diagnosis of brucellosis in some studies,[63,95,116,117] results have not been universally reproducible,[86,118,119] suggesting that the bacteraemia is as unpredictable as clinical manifestations in human brucellosis. Identification of *Brucella* strains is done using standard classification tests, including Gram stain, a modified Ziehl-Neelsen (ZN) stain, growth characteristics, oxidase activity, urease activity, H_2S production (four days), dye tolerance such as basic fuchsin (1: 50000 and 1: 100000) and thionin (1:25000, 1:50000 and 1:100000) and seroagglutination. Mantur and colleagues[63] have recommended Gram stain morphology and modified ZN staining, coupled with the urease test, for rapid identification of *Brucella* to the level of genus where facilities for further identification are not available. To the best of our knowledge, there is only one

report[120] suggesting antigen detection by enzyme-linked immunosorbent assay (ELISA) as an acceptable alternative to blood culture for the diagnosis of brucellosis since sensitivity and specificity were 100% and 99.2% respectively. Antigen detection methods are potentially useful but have not been validated. Though co-agglutination has been reported as a technique for antigen detection, there seems to be paucity of published literature. Laboratory detection of *Brucella* and species identification is based largely on culture isolation and phenotypic characterization. This process is lengthy and labour-intensive and has been associated with a heightened risk of laboratory-acquired infections. To surmount these problems, nucleic acid amplification has been explored for the rapid detection and confirmation of *Brucella*.

Polymerase chain reaction (PCR) is fast and can be performed on any clinical specimen.[121] A number of nucleic acid sequences have been targeted for the development of *Brucella* genus-specific PCR assays, including 16S rRNA, the 16S-23S intergenic spacer region, *omp2* and *bcsp31*.[122-125] Recently, Redkar *et al.*[126] described real-time PCR assays for the detection of *B. abortus, B. melitensis and B. suis* biovar1. These PCR assays target the specific integration of IS*711* elements within the genome of the respective *Brucella* species or biovar. Currently, a real-time multiplex PCR assay has been developed for rapid confirmatory identification of *Brucella* with speciation. The genus, *B. abortus* and *B. melitensis* specific primers confirm the organism from isolates.[127-128] One case of neurobrucellosis was confirmed in our laboratory with the CSF being positive by PCR but undetectable from the blood. The agglutinins were positive in CSF and blood. However, culture of blood and CSF was negative showing the utility of molecular methods in tertiary care centres. Molecular characterisation techniques described in the literature are very useful tools for differentiating *Brucella* spp. especially follow-up testing of unusual phenotypic results of *Brucella* isolates. Although PCR is very promising, standardization of extraction methods, infrastructure, equipment and expertise are lacking and a better understanding of the clinical significance of the results is still needed.[129] The use of molecular methods in *Brucella* endemic areas needs to be explored before they can be applied in these areas to diagnose brucellosis.

Unequivocal diagnosis of brucellosis requires isolation of the causal agent. Blood culture is the method of choice, but specimens need to be obtained early prior to antibiotic administration and need prolonged periods of incubation. In addition, failure to detect the pathogen is a frequent occurrence. Although in the last few years PCR-based laboratory tests have been proposed, they cannot be considered a routine diagnostic method yet. These limitations make serology for antibody detection the most useful tool for the laboratory diagnosis of brucellosis. Antibodies usually begin to appear in the blood at the end of the first week of the disease, IgM appearing first followed by IgG. The serological tests like Rose Bengal Plate Agglutination Test (RBPT), standard tube agglutination test (SAT), Coombs test, immunocapture agglutination test,[130] latex agglutination, complement fixation test, ELISA, lateral flow assay-a simplified version of ELISA, dipstick assay, fluorescence polarization assay (FPA),[131] have all been applied in the diagnosis of human brucellosis. The RBPT is often used as a rapid screening test.[132] The sensitivity is very high (>99%) but the specificity is disappointingly low.[63,133-134] However, this is of value as a screening test in high risk rural areas where it is not always possible to perform the tube agglutination titration test. To increase the specificity and the positive predictive value of the RBPT, the test may be applied to a serial dilution (1:2 through 1:64) of the serum sample. The specificity of the test increases when higher dilutions agglutinate and titres of 1:8 or 1:16 and above may be regarded as positive.[135] This approach may result in a lower sensitivity. Whenever possible, a serum that gives a positive result should be confirmed by a more specific test. The RBPT is also of value in the rapid confirmation of neurobrucellosis, arthritis, epididymoorchitis, hydrocele due to *Brucella* if the neat is positive in CSF, synovial fluid, testicular fluid /semen and hydrocele fluid respectively.[63,78]

SAT developed by Wright and colleagues[4] remains the most popular and yet used worldwide diagnostic tool for the diagnosis of brucellosis because it is easy to perform, does not need expensive equipments and training. SAT measures the total quantity of agglutinating antibodies (IgM and IgG),[136] the quantity of specific IgG is determined by treatment of the serum with 0.05M 2-mercaptoethanol (2ME), which inactivates the agglutinability of IgM. SAT titres above 1:160 are considered diagnostic in conjunction with a compatible clinical presentation. However, in areas of endemic disease, using a titre of 1:320 as cutoff may make the test more specific. The differentiation in the type of antibody is also important, as IgG antibodies are considered a better indicator of active infection than IgM and the rapid fall in the level of IgG antibodies is said to be prognostic of successful therapy.[137] A survey conducted by Almuneef and coworkers[138] in 2002 in Saudi Arabia found various levels of SAT antibodies in many clinically cured patients. Recently, Mantur and colleagues[63] followed-up 79 patients diagnosed as having active brucellosis for different lengths of time and monitored for *Brucella* antibodies by SAT and 2ME agglutination. In most cases, *Brucella* SAT titres remained measurable, in spite of falling to low levels (Figure) ranging from 1:160 to 1:640 (diagnostic titres), despite an effective therapy and clinical cure. A remarkable finding of the study was that there was a sustained drop in 2ME titres in 97.5% of cases (Figure), reflecting the importance of the 2ME test for diagnosis of brucellosis in conjunction with the SAT, as well as for follow up brucellosis in *Brucella*-endemic countries. Gazapo *et al.*[139] claimed that ELISA was an excellent method for follow up of brucellosis; however, the results of our study[63] clearly indicate that the 2ME agglutination test is a useful assay, as it is inexpensive and

technologically simple with stable reagents. Coombs test that detects incomplete antibodies and immunocapture-agglutination tests have shown similar performance with higher sensitivity and specificity in the diagnosis of human brucellosis, both in the first stage of the disease and in cases with long evolution as well as in relapses and reinfections.[130] Indirect enzyme-linked immunosorbent assay typically uses cytoplasmic proteins as antigens. ELISA measures IgM, IgG and IgA, which allows for a better interpretation of the clinical situation. A comparison with the SAT, ELISA yields higher sensitivity and specificity.[140] ELISA is also reported to be the most sensitive test for the diagnosis of CNS brucellosis.[95,141-142] Among the newer serologic tests, the ELISA appears to be the most sensitive; however, more experience is needed before it replaces the SAT as the test of choice for brucellosis. A dipstick assay[143] offers a rapid and reliable diagnostic alternative in acute brucellosis. The rapid and simple assays like *Brucella* IgM and IgG lateral flow[144] and latex agglutination[145] have been developed recently. The sensitivity and specificity of lateral flow assay for culture confirmed brucellosis is >95%. The sensitivity of the latex agglutination assay was determined to be 89.1% for the initial serum samples collected for the patients with culture confirmed brucellosis and the specificity was 98.2%. Both these tests are ideal for use as field tests in remote areas and as point of care tests in hospitals and health care centres that lack the expertise and facilities to perform the more demanding classic serologic tests. Routine serological surveillance is not practiced even in *Brucella*-endemic areas and we suggest that this should be a part of laboratory testing coupled with a high index of clinical suspicion to improve the level of case detection. It is important to realize that household members of index cases of acute brucellosis may have been exposed to the pathogen as well and have become infected and ill.[62-63] Therefore, we also recommend for the routine screening of family members of index cases of acute brucellosis in an endemic area.

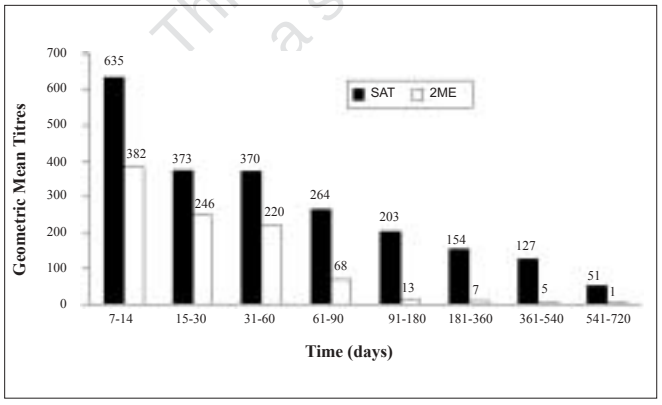

Figure: Results of the SAT and 2ME tests at different follow-up times in 79 cases. In most cases, in spite of falling to low levels, *Brucella* SAT titres remained measurable with significant titres despite an effective therapy and clinical cure, but there was a sustained drop in 2ME titres in 97.5% (77 / 79) of cases

Treatment and Prevention

The prerequisites for an effective therapy are: treatment should start on time, should consist of combination of drugs along with at least one drug having a good penetration into cells and should be prolonged. It seems advisable to follow-up the cases to assess the response to therapy as a guide for treatment with the help of either 2-ME or ELISA tests. The treatment of human brucellosis is a controversial area because of the spectrum of disease, the possibility of chronic infection and the development of complications.[146] Many antimicrobial agents are active against *Brucella* species; however, clinical efficacy does not always correlate with *in vitro* susceptibility.[147] In all cases it is important that the patient completes the full course of therapy because the risk of incomplete recovery and relapse is otherwise increased considerably.[148] The treatment recommended by the World Health Organization for acute brucellosis in adults is rifampicin 600 to 900 mg and doxycycline100 mg twice daily for a minimum of six weeks.[17] Some still claim that the long- established combination of intramuscular streptomycin (1 g/day for two-three weeks) with an oral tetracycline (2 g/day for six weeks) gives fewer relapses.[63,149] Trimethoprim-sulfamethoxazole is a popular compound in many areas, usually used in triple regimens. Quinolones are an alternative. Various combinations that incorporate ciprofloxacin and ofloxacin have been tried clinically, yielding similar efficacy to that of the classic regimens.[150] Although the results are encouraging, additional experience is needed in order to determine the role of fluoroquinolones in the treatment of brucellosis.[151] Childhood brucellosis can be successfully treated with a combination of two drugs; doxycycline 4 mg / kg / day and rifampicin 10 mg/ kg /day orally for six weeks.[78] Some authors advise that gentamicin (5 mg/kg/day intramuscularly) be administered concomitantly for the initial five to seven days of therapy in order to prevent relapse.[78,147] Co-trimoxazole (TMP/SMX) 8 mg / 40 mg/kg/day can be used for children < 6 years of age. Rifampicin with or without a combination of cotrimoxazole has proved safe to treat brucellosis during pregnancy.[83,152] Relapses occur at a rate of about 10% and are often milder in severity than the initial disease and can be treated with a repeated course of the usual antibiotic regimens.[83] Most complications of brucellosis can be adequately treated with standard regimens. Treatment of some complications like spondylitis, osteomyelitis, neurobrucellosis and endocarditis also require combination therapy but longer courses. For neurobrucellosis, combination therapy with two or three drugs - that is doxycycline, rifampicin and trimethoprim-sulfamethoxazole that penetrate CNS and are active against the infecting isolate is recommended.[141] The combination of doxycycline with rifampicin and trimethoprim-sulfamethoxazole has been used successfully in the treatment of brucellar endocarditis.[141] Although cases of endocarditis caused by *Brucellae* have been cured with antimicrobial chemotherapy alone,[63,78,153,154] it is generally

believed that surgical intervention (valve replacement) combined with antibiotic therapy is the best approach.[16]

Prevention of human brucellosis is dependent on control of the disease in domestic livestock mainly by mass vaccination.[155] In many countries, the use of *B. abortus* strain vaccine in cattle and *B. melitensis* strain Rev-1 vaccine in goats and sheep has resulted in the elimination or near-elimination of brucellosis in these animals. Studies are ongoing to develop an effective vaccine against *B. suis*. Since the treatment of animal brucellosis is very expensive, one should encourage the mass vaccination of livestock. Animal owners should be taught about the importance of vaccination of their animals. In spite of the clinical efficacy and cost effectiveness of vaccination, the limited availability of vaccines and lack of awareness has led to the persistence of brucellosis in most areas including India. The lack of human vaccines and effective control measures make it necessary for the doctors and other health care workers to take protective measures. Protective clothing / barriers while handling still births / products of conception and cultures can reduce occupation-related brucellosis.[16,93] The avoidance of unpasteurised dairy products will prevent infection in the general population.[156]

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