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Brucellosis: An Economically Important Infection

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Abstract

Brucellosis is one of the world's major zoonosis, caused by bacteria of the genus *Brucella*. The world's most widespread zoonosis affects cattle, sheep, goats, pigs, and other animals, leading to abortion, infertility, and low milk yields. Humans acquire brucellosis from direct contact with livestock or from drinking unpasteurized milk. *Brucella* spp. are considered as the most common laboratory-acquired pathogens. Several serological tests have been widely used for diagnosis of *Brucella* such are Rose Bengal plate test (RBPT), Standard tube agglutination test (STAT), complement fixation test (CFT), enzyme linked immunosorbant assay (ELISA). Besides these, polymerase chain reaction (PCR) based identification and typing, fluorescence polarization assay (FPA) are also important diagnostic tools. The worldwide economic losses due to brucellosis are extensive. Although a number of successful vaccines are being used for immunization of animals still no satisfactory vaccine against human brucellosis is available. This review shows world literature and its impact to the history, epidemiology, virulence, diagnosis along with the control measures adopted in all over the world scenario including Indian.

Keywords: Brocellosis; Brucella; Zoonosis; Serological tests

Introduction

Impact on health and economy

Brucellosis is a highly infectious zoonotic disease and an economically important infection of humans and livestock with a worldwide distribution. It is a major veterinary and human public health problem in most parts of the world. The incidence of this disease is greatly decreased in the developed world due to effective vaccination based control programs, but remains an uncontrolled problem in regions of high endemicity such as the Mediterranean, Middle East, Africa, Latin America and parts of Asia including India [1-3]. Across the developing world, brucellosis is still a very common but often neglected disease. Brucellosis is of economic concern in many parts of the world as it results in reduced productivity, abortions, weak offspring's and major impediments for trade and export of livestock. It can also be transferred from animal to humans [4]. Brucellosis is a chronic disease with a risk of disabling consequences, but is rarely fatal in affected humans. Human brucellosis is a severe debilitating disease that requires prolonged treatment with the use of several antibiotics and also involves considerable medical expenses as well as loss of working hours. Brucellosis is almost invariably transmitted to man from infected domestic animals. However, it has been documented beyond doubt, the possibility of human to human transmission of *Brucella* infection [5-7] i.e. humans carry the disease, but person to person transmission of brucellosis is very rare, however transmission of the disease from human to human has been reported [8-10]. Mothers who are breast-feeding may transmit the infection to their infants and sexual transmission has also been reported [10,11].

Besides a threat to human healthware brucellosis spread in livestock foci is also causing serious problems to the national economies. According to the International agreements on the veterinary regulation [12] if brucellosis is detected in at least one herd, the resettlement and sale of animals from the whole foci region should be prohibited. Such strict limitations lead to the significant brucellosis mediated economic losses.

Marston described the symptoms of brucellosis and also gave the name gastric remittent fever [13]. Brucellosis has many synonyms derived from the geographical area in which this disease is common, e.g. Mediterranean fever, Malta fever, Gibraltar fever, Cyprus fever. It was also known with the symptoms it is associated, undulant fever due its remittent character and typhomalarial fever due to its reassemble to malaria and typhoid fevers. That is why brucellosis is frequently misdiagnosed as malaria, typhoid, or venereal disease [14]. Brucellosis is also known as intermittent typhoid, bang's disease in cattle, contagious abortion, infection abortion, epizootic abortion.

This disease has been under reported from domestic animals from developing countries because of absence of national surveillance programs, diagnostic facilities and reliable data [15]. The principal symptom in all animal species is abortion or premature expulsion of the fetus. The main mode of transmission of this disease to humans is through consuming untreated milk products. Each year about a half million cases of brucellosis occurs in humans around the world [16]. There are three reports of humans infected with marine strains of *Brucella*; one reported in a research laboratory worker after occupational exposure [17] and other two were community-acquired infections [18,19]. Bovine brucellosis has been eradicated in Finland, Norway, Sweden, Denmark, Belgium, Switzerland, Germany, Austria, Hungary, the former Czechoslovakia, Rumania, and Bulgaria, as well as in other developed countries [20,21].

Historical perspective

Marston made the earliest recorded description of brucellosis in 1859 as he wrote of an illness, including his own, which differed from typhoid fever. Sir David Bruce isolated the organism from the spleen of a patient while investigating an outbreak of a fatal disease known as Mediterranean or Malta fever, affecting British soldiers stationed on the island of Malta [22]. He named the bacteria as Micrococcus

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melitensis due to coccoidal morphology. Hughes suggested the name undulant fever (wave like) because of characteristic fever, which rise and fall over weeks in untreated patients [23]. Write and Smith detected antibodies of *M. melitensis* through agglutination test in humans and explained the zoonotic potential of this disease [24] Zammit working with Mediterranean fever commission discovered the role of goats in brucellosis by isolation the organism from the milk and urine of the goats and concluded that goat was the reservoir and declared that consumption of the raw milk and cheese responsible for the human brucellosiss [25]. The report of isolation of a gram negative rod from cattle, its subsequent establishment of similarity between *M. melitensis* gave convincing evidence that both organisms could not be differentiated morphologically or by cultural and biochemical reactions. Both these bacteria were finally placed under one genus *Brucella* named in honour of Sir David Bruce.

Classification and General Characteristics

Scientific classification

Kingdom- Bacteria

Phylum-Proteobacteria

Class-Alphaproteobacteria

Order-Rhizobials

Family-Brucellaceae

Genus-*Brucella*

The traditional taxonomy is based on phenotypic characteristics, antigenic variations and prevalence of infection in different animal hosts. The common species of *Brucella* associated with different animal hosts are *B. melitensis* (goat and sheep), *B. abortus* (cattle), *B. suis* (pig, reindeer and hare), *B. ovis* (sheep), *B. neotomae* (desert wood rat), and *B. canis* (dog). *B. pinnipediae* (seal/ otter) and *B. cetaceae* (porpoise/ whale) have been reported from marine mammals [26,27]. Which of them human infections are common with B. *melitensis* [2,26]. *B. abortus* as α2 proteobacteria have phylogenetic relationships with Agrobacterium, Rickettsia, Rhizobium, Rhizobacter, Ochrobacterium [28,29]. *Brucella* has been subdivided into biovars based on different biochemical reactions and differentiated from other related species by conventional methods such as sensitivity or tolerance to aniline dyes, production of H_2S and CO_2 requirements for growth. The taxonomy of *Brucella* species is still being resolved based on 16s-rRNA gene sequence. According to the new taxonomy used by NCBI the species *B. melitensis* includes 5 biovars namely, *abortus, canis, neotomae, ovis, and suis* (Table 1).

Brucellosis in animals

Brucellosis is a very important disease for bovines and buffaloes due to the reproductive problems it causes [30] and also the risk to public health. It is a barrier to the international trade of animals and animal products [31]. The main pathogen is *B. abortus* biovar 1 is universal in the presence and predominant among the seven that occurs in the world. The distribution of the different biovars varies geographically. Cattles can also become infected with *B. suis*, *B. melitensis* when they share pasture or facilities with infected pigs, goat and sheep. The infections in cattle caused by other species of *Brucella* are more sporadic and rarer in nature than the disease caused by *B. abortus*. In natural infections it is difficult to measure the incubation period (from time of infection to abortion or premature birth), as it is not possible to determine the moment of infection. Experiments have shown that the incubation period varies considerably and is inversely proportional to fetal development, i.e. the more advanced the pregnancy, the shorter the incubation period. If the female is infected orally during the breeding period, the incubation period can last up to 200 days, while if she is exposed six months after being bred, the incubation time is approximately two months. The period of "serologic incubation" (from the time of infection to the appearance of antibodies) lasts several

Taxonomic characteristics of *Brucella* species								
Species	**Bio- type**	**Host reservoir**	**Biochemical identification**					
			Fuchsin	**Thionin**	**Safranin -inhibition**	**H_2S production**	**Urease**	**CO_2 growth**
B. mlitensis (Bruce, 1887)	1-3	Goats, sheep, camels	+	+	-	-	+ in 24 hr.	-
B. abortus (Bang, 1897)	1-6,9	Cows, camels, yaks, buffalo	+ (except biotype 2)	- (biotype 1, 2, 4)	-	+ (except biotype 5)	+ in 24 hr.	+
B. suis (Traum, 1914)	1-5	Pigs (biotypes 1-3), wild hares (biotype 2), Caribou (biotype 4), reindeer (biotype 4), wild rodents (biotype 5)	- (except biotype 3)	+	+	+ (biotype 1)	+ in 15 min.	-
B. canis (Carmichael and Bruner, 1968)	--	Canines	+ / -	+	-	-	+ in 15 min.	-
B. ovis (Van drimmelen, 1953)	--	Sheep	- for some strains		-	-	-	+
B. neotomae (Stoenner and Lackman, 1957)	--	Rodents	-		-	+	+ in 15 min.	-
B. pinnipediae* and *B. cetaceae (Ewalt et al., and Ross et al., 1994)	--	Mink whales, dolphins, porpoises (pinnipediae), seals (cetaceae)	+	+	-	-	+	- for pinnipediae and + for cetaceae.

Table 1: Taxonomic characteristics of *Brucella* species.

weeks to several months. The incubation period varies according to factors such as the virulence of the strain and dose of bacteria, the route of infection and the susceptibility of the animal. In short, incubation period varying from 5 days to 5 months and can progress in various forms: acute, chronic or asymptomatic [32].

In pregnant females, abortion occurs during the second half of the pregnancy, often with retention of the placenta and resultant metritis, which may cause permanent infertility. It is estimated that the infection causes a 20% to 25% loss in milk production as a result of interrupted lactation due to abortion and delayed conception. In bulls it may become localized in the testicles and adjacent genital glands. The bacteria enter the body of animals first multiply in the regional lymph nodes and the latter carried by the lymph and blood to different organs. In experimental infection, it is possible to isolate the agent from the bloodstream after two weeks of infection. *Brucella* organisms are most commonly found in the lymph nodes, uterus, udder, spleen, liver, and in bulls, the genital organs. Large quantities of erythritol, a carbohydrate that stimulates the multiplication of brucellae, have been found in cow placentas and this could explain the high susceptibility of bovine fetal tissues. The virulence of *Brucella* in cattle is mainly due to their ability to replicate intracellularly, preferentially utilize erythritol and inhibit the mature reproductive tract, which is high in this sugar [33]. Once an infected cow aborts or gives birth normally, the pathogen does not remain long in the uterus. The infection becomes chronic and the brucellae are harboured in the cow's lymph nodes and mammary glands [34].

Cows, especially when pregnant are most susceptible, although some researchers maintain that bulls are more resistant to the infection than females. Some less susceptible cows have generalized infections and suffer losses in the reproductive system and milk production for one or more years, but then gradually recover. In such animals, the agglutination titer become negative and both the reproductive system and milk production return to normal. However, most cows become infected and their agglutination titers remain positive for many years. Brucellosis spread rapidly from animal to animal, trading and movements of animals also help maintain active infection.

Brucellosis in humans

Brucellosis is a multi-systemic disease in human and may present with a broad spectrum of clinical manifestations and its complications can affect almost all organs and systems with varying incidence [35-37]. It is fatal in 1%–5% of untreated cases [38,39]. The symptoms and clinical signs most commonly reported are fever, fatigue, malaise, sweats, headaches, myalgia, arthalgia and weight loss [40]. Some cases have been presented with only joint pain, low back ache, and involuntary movements of limbs, burning feet or ischemic heart attacks [41]. Human brucellosis usually manifests as an acute (less than two months) or sub-acute (2-12 months) febrile illness, which may persist and progress to a chronic stage referred as chronic fatigue syndrome [42]. Complications can be different depending on the specific site of infection [43] of which meningitis and meningoencephalitis is the most common complications seen in neurobrucellosis [44]. Neurobrucellosis has been reported as an exceptional cause of transient ischemic attacks [41]. In conclusion, it should be noted that brucellosis may affect essentially any organ and that reinforces the importance of brucellosis in differential diagnosis in endemic areas [45] (Figure 1).

Mode of Transmission

The disease is transmitted either through contaminated milk products or through direct contact with infected animals. Fresh milk and dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts and ice creams may contain high amounts of the bacteria and consumption of these is an important mode of transmission to humans [46]. It is also estimated that 10-100 organisms in aerosol form constitute an infectious dose. The bacterium may enter the body through digestive tract, the lungs or mucosal layer and spread through the blood and lymphatic system to any other organ where it may infect the tissue and cause localized. This is a common laboratory transmitted infection and reported to occur in clinical, research and production laboratories [47,48]. Bacterial load in animal muscle tissue is low, but consumption of undercooked traditional delicacies such as the liver has been implicated in human infection [49]. The main sources of infection for cattle are fetus and vaginal discharges. The most common route of transmission is the gastrointestinal tract following ingestion of contaminated pasture, feed, fodder, or water. Moreover, animals customarily lick afterbirth fetuses and newborn calves, which contain a large number of the bacteria. It has been shown experimentally that the organism may penetrate broken and even intact skin, but the mode of transmission of natural infection is unknown. Vaginal route and intrauterine route used in artificial insemination are very important in transmitting the infection.

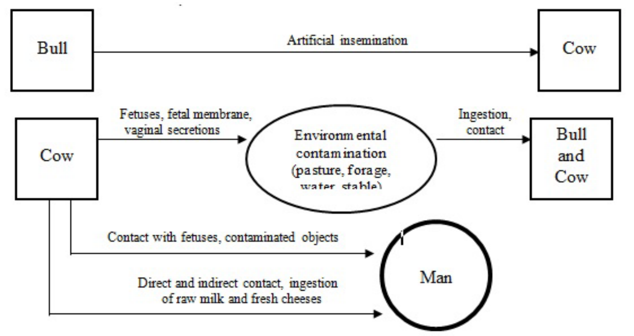

Figure 1: Brucellosis - Mode of transmission bovine brucellosis (Pedro and Boris, 2001).

Global scenario

The epidemiology of brucellosis is complex and Latin American countries, mainly Mexico and Peru reported a large number of cases. The same pattern holds true for Mediterranean countries like Iran, former Soviet Union and Mongolia. Seven republics of the former Soviet Union (Kyrgyzstan, Tajikistan, Kazakhstan, Azerbaijan, Turkmenistan, Armenia and Uzbekistan) are included in the 25 countries with the highest incidence of the disease worldwide, while another country of this region, Mongolia, is ranked the second. Syria has the highest annual incidence of human brucellosis worldwide [50]. Human brucellosis is found to have significant presence in rural/nomadic communities of these countries 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 100000 population [51] and about 500000 cases/year of human brucellosis are reported worldwide, but the estimates of the World Health Organization (WHO) suggest that, due to underreporting, the real incidence is 10–25 times higher [52]. Despite the fact that incidence of human brucellosis is unknown for most countries including India and no data are available [53].

In Saudi Arabia, 7893 human cases of brucellosis were recorded in 1987 (74 per 100000 inhabitants). In Iran, 71051 cases (13 per 100000) were recorded in 1988 and it is estimated that 80000 cases have occurred each year since 1989. In turkey 5003 cases (9 per 100000) were recorded in 1990, an incident three times higher than the period 1986-1989 (3 per 100000).

In Europe, brucellosis is declining, according to data from the European Food Safety Authority (EFSA) the number of cases decreased from 735 in 2008 to 352 in 2011 [54]. The disease affects mostly the Mediterranean countries. From 2008 to 2011 Greece, Italy, Portugal and Spain accounted for 50–80% of all the European reported cases, respectively, with *B. melitensis* and *B. abortus* being the predominant causative agents [54].

The large meat producing countries such as France, Great Britain, Australia, New Zealand, Canada and United States are free of bovine brucellosis. The three important cattle raising countries, Argentina, Brazil and Mexico, still have limited control programs. A country-by-country analysis is found in a monograph on bovine brucellosis [34]. In the rest of the world, rates of infection vary greatly from one country to another and between regions within a country. Official estimates put annual losses from bovine brucellosis in Latin America at approximately US$ 600 million [55]. A trend in the epidemiology of human brucellosis in Germany was investigated by analyzing national surveillance data (1962–2005) complemented by a questionnaire- based survey (1995–2000). The incidence decreased from 1962 to the 1980s' even though a persistent number of cases have been reported among Turkish immigrants (0.3/10000 Turks vs. 0.01/100000 in the German population [56].

Indian scenario

India has a huge resource of livestock and dairy farming plays a substantial role in the country's rural economy [57]. The country restrains the largest buffalo population in the world (105.34 million - 57.3%) followed by the 2nd largest cattle population (199.08 million - 14.7%) [58] and highest milk production in the world, i.e. 121.8 million tonnes with per capita availability of 281 g/day [59]. Brucellosis is a highly contagious disease of dairy animals and humans in many parts of the world, including India causing significant morbidity and enormous economic losses [60,61]. The disease causes abortions in the last trimester of pregnancy, premature births followed by retention of placenta, metritis, decreased milk production and lameness as a common sequel to infection in dairy animals [62].

The occurrence of brucellosis in India was first established early in the previous century and since then has been reported from almost all states [63,64]. Many publications indicate that brucellosis is a fairly common disease in India and present in different species of mammalian farm animals including cattle, goats, buffalo, yaks, camel, horses and pigs [65-67]. A national survey in bovines a decade back indicated 5% of cattle and 3% of buffaloes of the country were infected with brucellosis [64]. The occurrence of the disease is usually high in organized farms (50%) compared to the marginal herds (10%) and this primarily associated with intensive farming practices in large organized animal farms [53,68] reported 8.5% seroprevalence of brucellosis among the dairy persons with the isolation of *Brucella* strain from seven cases of human brucellosis. As many as 4.2% aborted women were seropositive for disease [69].

In Gujarat, 8.5% [70] and in Hariyana, 34% human brucellosis cases were reported among veterinarians, attendants and compounders who are in contact with animals [71]. In a study conducted by Hemashettar and Patil 24 (8.2%) veterinary workers showed *Brucella* specific antibodies in significant titers [72]. A study by Mantur and coworkers in Bijapur reported 93 children among 5726 children as seropositive by SAT (>1:160) and confirmed it by the isolation of B. melitensis in 43 pediatric patients [73]. Handa and coworkers identified four cases with acute brucellosis in a group of 121 patients with FUO (Fever of Unknown Origin) [74].

Genome

The genome contains 2 circular chromosomes except *B.suis* biovar 3, which has a single chromosome. The size of the first chromosome of *B. abortus* is 2,124,241 nucleotides long and codes for 2200 genes. The second chromosome is 1,162,204 nucleotides long and codes for 1156 genes. The genome has a GC content of 57%, and 81% of the genome is coding region [75]. This pathogen is different from other bacterial species as it does not contain any plasmid or genomic islands that related to pathogenicity within its genome. In addition to lacking these two features, the genome also lacks many other genes that code for common virulence factors, including "capsules, fimbriae, exotoxins, cytolysins, resistance forms, antigenic variation, plasmids, or lysogenic phages" [76]. The genes that do encode for virulence in *B. abortus* are being examined, but they are not well understood to say for sure the mode of the virulence of this intracellular pathogen [26].

Virulence and Pathogenicity

There are many factors which responsible for human brucellosis. The S-LPS is a major determinant of virulence and dominants the antibody response. The elimination depends on activated macrophages and hence requires the development of Th1 type cell mediated immunity. *Brucella* LPS is a relatively poor inducer of gamma interferon and tumor necrosis factor α, both of with are essential for the elimination of the organism [77]. The other important virulence factors include, production of inhibitory phagolysosome fusion such as adenine and guanine monophosphate levels [78]; outer membrane protein 25 which has been identified as the down regulator of TNF α [79] especially in the early stage of infection. Recently ureas enzyme has been identified as an important determinant of virulence as the areas enzyme protects bacteria in their passage through the stomach by oral route, which is the major way of infection in human brucellosis. *Brucella* is also considered as Class III pathogen and listed as a potential bio-threat agent that can be used in bioterrorism.

Laboratory Diagnosis

The varied symptoms which brucellosis presents make it troublesome for clinical diagnosis. The conventional diagnosis is microbiological confirmation by means of isolation of bacteria from the blood or from other body fluids. The isolation rate of *Brucella* is poor due to its slow growth rate, quantity of circulating viable bacteria, culture medium, blood culture techniques employed as well as presence of antibiotics that inhibits growth [80]. The demonstration of antibodies generated against *Brucella* by serological tests remains a viable alternative to culture and several serological tests like Standard Tube Agglutination Test (SAT) and Rose Bengal Plate Agglutination Test (RBPT) are the most popular serological tests used in the field for the diagnosis of brucellosis. Several workers have reported development of antibody detection systems based on ELISA.

Blood culture provides definite proof of brucellosis [81], but may not provide a positive result for all patients even under ideal conditions. *Brucella* is a slow growing organism and cultures are rarely positive and should be kept at least 45 days before the culture can be concluded negative. Many serological tests have been used for the diagnosis of brucellosis. The most commonly used tests are serum agglutination test

(SAT), the coombs anti *brucella* test, rose bengal plate agglutination test (RBPT, based on agglutination of colored particulate antigen (killed *Brucella* organisms) by the antibodies present in the patient's serum), complement fixation test (CFT), indirect heamolysis test (IHLT). Since the development of the first agglutination test of brucellosis by Wright and Smith in 1897, veterinary laboratory workers have been developing tests to improve diagnostic performance and accuracy. Among the various tests developed are rapid agglutination tests for the detection of antibodies to brucellosis in cattle sera, such as the Rose Bengal Test [82], the Card Test [83], and the Buffered Antigen Plate Agglutination Test (BPAT) [84]. These tests use acidified antigens and were developed to improve accuracy. The purpose of the acidified antigens was to reduce agglutination by IgM, thus reducing nonspecific false-positive reactions. Although rapid, these tests were largely laboratory based and subjective in the interpretation of results. With the exception of the BPAT, they did not significantly improve test accuracy [85]. The SAT detects IgG less efficiently, especially IgG1, resulting in low assay specificity [86-88]. Therefore, the SAT is generally not used as a single test, but rather in combination with other tests.

In 1897, Wright and Smith published the first description of a test for the serological diagnosis of brucellosis in man. After that, different diagnostic tests are developed and there is a need to improve them. Dot-enzyme linked immunosorbent assay (dot-ELISA) for the detection of *Brucella* antibodies in human sera with autoclaved extract of *B. abortus S99* was developed and results were compared with those of STAT, RBPT and CFT. The dot-ELISA was found to be a more sensitive and also economical and rapid test for screening of human brucellosis under field conditions [89,90] evaluated a dot-ELISA (d-ELISA) test with the serum agglutination test (SAT), micro-complement fixation test (CFT) and a plate-ELISA (p-ELISA) for field use in screening herds of goats against brucellosis and found that d-ELISA was more suitable and rapid test for screening large numbers of goats in the field. YasminB and Selvam DT [91] found that d-ELISA formate had a high correlation, sensitivity and specificity in comparison with RBPT and plate ELISA. According to Shome et al., [92] the lateral flow assay (LFA) is a cost-effective and rapid technology that provides accurate detection of antibodies to *B. abortus* in bovine serum samples.

Brucella antibodies in bovine sera and milk was also detected using the dot-immunobinding assay (DIA), the serum agglutination test (SAT), the Rose Bengal plate test (RBPT) and the milk ring test (MRT). In DIA, *B. abortus S99* antigen prepared by heat treatment was used [93].The efficiency of a single antigen as well as a combination of two antigens in the complement fixation (CF) test was determined in detecting cattle and sheep infected or vaccinated. Comparative analysis of the CF results showed that the combined S99/RB51 antigen used in the CF test increases the specificity and sensitivity and could be used in animal brucellosis surveillance [94].

The humoral immunoresponse to S brucellae is dominated by antibodies to the PS (polysaccharide) section of the *Brucella* S-LPS (smooth lipopolysaccharide) and it shows a typical IgM/IgG (and IgA) shift. S-LPS or PS tests proposed for the diagnosis of human brucellosis, recently include the lateral flow immunochromatography assay (LFiC) for IgM and IgG assessment, a fluorescence polarization assay, a variety of indirect ELISA, and the immunocapture Brucellacapt test [95]. In addition, a competitive ELISA (cELISA) has been proposed [96]. In acute cases (i.e., short evolution) IgM is present in the serum; then this immunoglobulin returns progressively to background levels, so that IgG (and IgA) are dominant in the sera of long evolution (i.e. chronic) patients before treatment.

Treatment, control and prevention

Uncomplicated acute brucellosis almost invariable responds well to appropriate antibiotic treatment [97,98]. Patients with complications, additional treatment, including in some cases surgical intervention will be necessary. To prevent disease progression and the development of complications, treatment should start as early as possible also in patients showing signs of spontaneous improvement. In all cases it is important that the patient finishes the full course of medication because the risk of incomplete recovery and relapse is otherwise increased considerably [99]. Either taking the combination of doxycycline and rifampicin (for 6 weeks), or the combination of doxycycline (100 mg twice/day orally for 6 weeks) with streptomycin (1 g/day for 2-3 weeks) is the standard treatment for brucellosis [100]. The effectiveness of the combination of streptomycin with a tetracycline has been acknowledged since the early days of antibiotic use [101], and the addition of rifampicin in treatment regimens for brucellosis also has a history of more than 30 years [102]. Treatment of complications such as spondylitis and osteomyelitis, neurobrucellosis and *brucella* endocarditis may require prolonged therapy for at least 8 weeks. Other combinations such as co-trimoxazole plus doxycycline and co-trimoxazole plus rifampin have been proposed, but still need further examination [103-106]. The optimal therapy for brucellosis during pregnancy has not been established [107].

The prevention of brucellosis is mainly by control of infection in domestic livestock by mass vaccination. The use *B. abortus* strain S19 in cattle and *B. melitensis* strain Rev-1 in goat and sheep has drastically reduced its incidence in many endemic areas. Vaccination of livestock is relatively cheap and will increase the value and productivity of their animals. It is not only important to improve the health of their animals but also is an important step to reduce the risk of severe illness and disability for themselves and their family members and also reduce the transmission to the human population. India already has developed a plan for the control of bovine brucellosis3 but the non-availability of a human vaccine makes it necessary for the animal handlers, doctors and health care workers take protective measures. The avoidance of unpasteurised dairy products will prevent infection in the general population.

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