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**Brucellosis in India: An overview****DJ Talukdar, Papori Talukdar, DK Murasing and A Kayina**DOI: <https://doi.org/10.22271/chemi.2020.v8.i3a.9802>**Abstract**

Brucellosis is one of the most important and widespread zoonotic disease in the world. Animals may transmit *Brucella* organisms during septic abortion, during slaughter, and through their milk. Brucellosis is rarely transmitted from person to person. The incidence of brucellosis in human is closely tied to the prevalence of infection in animals, and to practices that allow exposure of humans to potentially infected animals or their products. *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, and marine mammal *Brucella* species are human pathogens. *Brucellae* can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, and the gastrointestinal tract. Bison, elk, reindeer and caribou can be infected with *Brucella spp.*, but their role in transmission of the infections to livestock remains under debate. The uncomplicated cases should be treated with doxycycline, streptomycin and rifampicin. Mass vaccination is crucial for the control and eradication of bovine, ovine and caprine brucellosis. Complementary measures that may need to consideration like improved farm hygiene, restriction and control of trade and movement of animals, testing of animals and isolation and removal of infected animals.

**Keywords:** Brucellosis, India, prevalence, pathogenesis, clinical manifestation**Introduction**

According to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the Office International des Epizooties (OIE), brucellosis is still one of the most important and widespread zoonoses in the world. Infections are caused by various bacteria of the genus *Brucella*, which tend to infect a specific animal species. However, most species of *Brucella* are able to infect other animal species as well and some of them have zoonotic potential. In humans, brucellosis can be caused by *B. abortus*, *B. melitensis*, *B. suis* biovars 1-4 and, rarely, *B. canis* or marine mammal *Brucella*. *B. ovis*, *B. neotomae*, and *B. suis* biovar 5 have not been associated with human disease. The disease affects cattle, swine, sheep and goats, camels, equines, and dogs. It may also infect other ruminants and marine mammals. A new strain, named *Brucella microti*, was recently isolated from the common vole (*Microtus arvalis*) in Central Europe [1]. *Brucella pinnipediae* and *cetaceae* are also newly recognized marine mammal *Brucellae* that may also be human pathogens [2,3].

In 1887 David Bruce, for whom the genus *Brucella* is named, isolated the causative organism from the spleens of five patients who died from the disease and placed the microorganism within the genus *Micrococcus*. Ten years later, ML Hughes, who coined the name "undulant fever," published a monograph that detailed clinical and pathological findings in 844 patients [4].

That same year, Danish investigator B Bang identified an organism, which he called the "bacillus of abortion," in the placentas and fetuses of cattle suffering from contagious abortion. In 1917 AC Evans recognized that Bang's organism was identical to that described by Bruce as the causative agent of human brucellosis. The organism infects mainly cattle, sheep, goats, and other ruminants, in which it causes abortion, fetal death, and genital infections [5, 6]. Humans, who are usually infected incidentally by contact with infected animals or ingestion of dairy foods, may develop numerous symptoms in addition to the usual ones of fever, malaise, and muscle pain.

**Infectious agent**

*Brucellae* are small, nonmotile, nonsporulating, nontoxicogenic, nonfermenting, facultative, intracellular, gram-negative coccobacilli.

## Epidemiology

Animals may transmit *Brucella* organisms during septic abortion, during slaughter, and through their milk [7]. Brucellosis is rarely, if ever, transmitted from person to person. The incidence of human disease is thus closely tied to the prevalence of infection in sheep, goats, and cattle, and to practices that allow exposure of humans to potentially infected animals or their products. In the United States, where most states are free of infected animals and where dairy products are routinely pasteurized, illness occurs primarily in individuals who have occupational exposure to infected animals, such as veterinarians, shepherds, cattlemen, and slaughterhouse workers. In many other countries, humans more commonly acquire infection by ingesting unpasteurized dairy products, especially cheese.

## Pathogenesis

Brucellae can enter mammalian hosts through skin abrasions or cuts, the conjunctiva, the respiratory tract, and the gastrointestinal tract [8]. In the gastrointestinal tract, the organisms are phagocytosed by lymphoepithelial cells of gut-associated lymphoid tissue, from which they gain access to the submucosa [9]. Organisms are rapidly ingested by polymorphonuclear leukocytes, which generally fail to kill them [10, 11] and are also phagocytosed by macrophages. Bacteria transported in macrophages, which travel to lymphoid tissue draining the infection site, may eventually localize in lymph nodes, liver, spleen, mammary glands, joints, kidneys, and bone marrow.

In macrophages, brucellae inhibit fusion of phagosomes and lysosomes [12] and replicate within compartments that contain components of endoplasmic reticulum [13] via a process facilitated by the type IV secretion system [14]. If unchecked by macrophage microbicidal mechanisms, the bacteria destroy their host cells and infect additional cells. Brucellae can also replicate extracellularly in host tissues. Histopathologically, the host cellular response may range from abscess formation to lymphocytic infiltration to granuloma formation with caseous necrosis.

In ruminants, *Brucella* organisms bypass the most effective host defenses by targeting embryonic and trophoblastic tissue. In cells of these tissues, the bacteria grow not only in the phagosome but also in the cytoplasm and the rough endoplasmic reticulum [15]. In the absence of effective intracellular microbicidal mechanisms, these tissues permit exuberant bacterial growth, which leads to fetal death and abortion. In ruminants, the presence in the placenta of erythritol may further enhance growth of brucellae. Products of conception at the time of abortion may contain up to 10<sup>10</sup> bacteria per gram of tissue [16]. When septic abortion occurs, the intense concentration of bacteria and aerosolization of infected body fluids during parturition often result in infection of other animals and humans.

## Prevalence of brucellosis

### Global scenario

The brucellosis which has been known like Malta fever, undulant fever, Rock of Gibraltar fever and Bang's disease has very old history, as organisms resembling *Brucella* had been detected in carbonized cheese from the Roman era. Brucellosis was first recognized as a disease affecting humans on the Island of Malta in the early 20th century. Though its distribution is worldwide; yet brucellosis is more common in countries with poorly standardized animal and public health programme [17]. The routes of infection are multiple i.e., food-

borne, occupational or recreational, linked to travel and even to bioterrorism. New *Brucella* strains or species may emerge and existing *Brucella* species adapt to changing social, cultural, travel and agricultural environment [18]. The incidence of reactors in newly established cattle farms may be more than 30%, however, the highest rate (72.9%) of infection till now has been reported in the Palestinian Authority [19]. It is interesting to note that the second highest prevalence (71.42%) of brucellosis has been reported in mules from Egypt [20]. Invariably, all domestic animals suffer from this disease. Brucellosis in buffaloes has been reported from Egypt (10.0%) and Pakistan (5.05%) [20]. Since cattle are found through out the world, prevalence of brucellosis (0.85 to 23.3%) in cattle has been reported from a wide range of countries [21]. In camels, brucellosis has been reported from Arabian and African countries (0.0- 17.20%), where the disease also occurs in buffaloes, equines and swine [6]. Variable prevalence of this disease has been reported in sheep and goats. Bio varieties of *Brucella* vary with respect to geographic region. *Br. melitensis* biovar 1 from Libya, Oman and Israel and *Br. melitensis* biovar 2 from Turkey and Saudi Arabia have been isolated. *Br. melitensis* biovar 3 is the most commonly isolated species from animals in Egypt, Jordan, Israel, Tunisia and Turkey [21]. *Br. abortus* biovar 1 in Egypt, biovar 2 in Iran, biovar 3 in Iran and Turkey and biovar 6 in Sudan have been reported [22]. The countries with the highest incidence of human brucellosis include Saudi Arabia, Iran, Palestinian Authority, Syria, Jordan and Oman. Bahrain is reported to have no incidence [21]. The percent prevalence of bovine brucellosis has been reported to decrease in Ireland and Italy during the year 1999-2000 but there had been a trend towards a significant increase in Azores [23].

### Indian scenario

Brucellosis is an economically important disease of wild and domestic animals and it is particularly important in Indian context which is the largest producer of milk in the world and enriched reservoir of varieties of bovine species. Although it is very crucial for the economy of developing country like India, but regular monitoring of brucellosis in animals have received no attention till date. Bovine brucellosis is widespread in India and appears to be on the increase in recent times, perhaps due to increased trade and rapid movement of livestock [24]. The natural service of the livestock in rural areas is perhaps yet another important factor in the maintenance and spread of infection. Free grazing and movement with frequent mixing of flocks of sheep and goats also contribute to the wide distribution of brucellosis in these animals. Chahota *et al.* [25] have reported a severe outbreak of brucellosis in an organized dairy farm leading to abortions, retained placenta and still birth in cows. Mathur [26] reported 8.5% sero-prevalence of brucellosis among dairy personnel in contact with infected animals. In a separate study carried out by Mathur [27] in Haryana, concluded the goats and sheep as the sources of human infection by isolating *B. melitensis* as a predominant strain from human blood as well as milk samples from goats and sheep. As many as 4.2% aborted women were seropositive for the disease [28]. In Gujarat, 8.5% prevalence of *Brucella* agglutinins was recorded in human cases [29]. The study conducted by Thakur and Thapliyal [30], revealed a prevalence rate of 4.97% in samples obtained from persons exposed to animals. The much higher seroprevalence rate has been also noted in specific risk groups such as abattoir workers [31, 32]. The study which was carried out by Bandyopadhyay *et al.*, [33]

in yaks of North-Eastern hilly yak tracts of Arunachal Pradesh of India to explore the seroprevalence of brucellosis in yaks and 23.79, 21.11 and 18.98% were found positive for brucellosis using avidin-biotin ELISA (AB-ELISA), Rose-Bengal plate test (RBPT) and standard tube-agglutination test (STAT), respectively. The alarming prevalence as recorded was highest among the yak cows (31.42%) followed by heifers (23.85%) and bulls (8.88%). Rajkhowa *et al.*,<sup>[34]</sup> studied on the seroprevalence of brucellosis in mithuns maintained at the National Research Centre on Mithun, Nagaland, India revealed that the number of animals found positive for brucellosis in avidin-biotin enzyme-linked-immunosorbent assay, standard tube-agglutination test and Rose-Bengal plate test were 34, 20 and 11%, respectively.

## Clinical manifestations of brucellosis

### Brucellosis in Cattle

Brucellosis in animals is generally typified by late-term abortions and inflammatory lesions in the male reproductive tract. Bovine brucellosis is usually caused by *B. abortus* which has seven different biovars, named 1, 2, 3, 4, 5, 6 and 9. Biovar 1 is the most important and widespread *B. abortus* biovar. Among 530 *B. abortus* samples isolated from humans and animals in Latin America from 1968 to 1991, 399 (75.3%) were biovar 1 and most of them were isolated from cattle<sup>[35]</sup>. Natural infection with other *Brucella* species is rare. In areas where *B. melitensis* infection is enzootic in small ruminants, it is rarely seen abortion in cattle even though some infected animals may become carriers and excrete the bacteria in the milk<sup>[6, 36]</sup>. Dafni *et al.*<sup>[37]</sup> described foci of *B. melitensis* infection in cattle in Israel, but the occurrence of abortion was not confirmed. *B. suis* infection in cattle is self-limiting and thus it is not sustained<sup>[38]</sup>.

Sexually mature females are more susceptible to *B. abortus* infection than bulls<sup>[6]</sup>. This susceptibility increases during pregnancy, and animals get more susceptible with the advance of pregnancy<sup>[39, 40]</sup>. *B. abortus* infection in calf is self-limiting<sup>[41]</sup>.

The period of incubation varies considerably and it is mainly influenced by gestation, exposure dose, age, and vaccination<sup>[6]</sup>. The length of the incubation period is inversely proportional to the stage of gestation at the time of exposure. Bang<sup>[42]</sup> observed an abortion in a three-month pregnant heifer 56 days after feeding her with the cotyledons from an aborting cow. Other experimental studies have shown incubation periods varying from 53 to 251 days<sup>[43]</sup>.

The major clinical sign in pregnant females is abortion in bovine and buffalo cows<sup>[40]</sup>. Abortion usually occurs from the 5th to the 8th month of gestation<sup>[36]</sup>. The occurrence of abortion is related to some factors, such as the stage of pregnancy, the number of infecting organisms and the animal resistance<sup>[6]</sup>. Bang<sup>[42]</sup> observed that infected pregnant females usually abort only once, and concluded that infected cows acquire immunity. Alternatively to abortion, premature, stillborn or weak calves may occur. Abortion is often followed by placental retention and metritis, which may cause permanent or transient infertility<sup>[44]</sup>.

Brucellosis does not usually result gross organic lesions<sup>[45, 46]</sup> but sometimes a mild interstitial inflammatory reaction in the mammary gland may be observed<sup>[47]</sup> which is associated with elimination of bacteria in the milk. Mammary gland macrophages may provide the intracellular environment for the persistence of *B. abortus* in the mammary gland of chronically infected cows<sup>[12]</sup>.

Despite being susceptible to the infection<sup>[48]</sup> no clinical signs have been observed in infected heifers at prepubescent stage. Edgington and Donham<sup>[49]</sup> reported that 15 heifers experimentally infected with *B. abortus* before breeding failed to abort during the subsequent pregnancy, but there are evidences that heifers infected before adulthood may abort and contribute to maintain the infection in the herd<sup>[36]</sup>.

The pathogenic action of *B. abortus* infection in bulls has long been established. Buck *et al.*<sup>[50]</sup> isolated it from bulls with seminal vesiculitis and/or orchitis. Infection of the reproductive tract may leads to orchitis, epididymitis, ampullitis and seminal vesiculitis. Orchitis is occasionally manifested, and when it occurs it is usually unilateral, but both testicles may be affected. Scattered foci of necrosis coalesce to produce total testicular necrosis<sup>[51]</sup>. Atrophy of testicle may also occur<sup>[52]</sup>. The syndrome involving seminal vesicles and ampoule is apparently more common than the one involving the testicles and epididymides<sup>[53]</sup>.

Brucellosis may result in fetal pneumonia, and to the naked eye the only recognizable lesion is a consolidation of the lungs associated with a grayish mottling in a certain number of fetuses<sup>[54]</sup>. Nevertheless not all the brucellosis aborted fetuses have pneumonia, and lung lesions are not specific enough to enable us to incriminate *B. abortus* as the cause of abortion<sup>[55]</sup>. Others gross lesions are also frequently described in aborted fetuses. Xavier *et al.*<sup>[47]</sup> observed that cows that were experimentally infected with *B. abortus* presented either weak newborn calves or aborted fetuses with mild peritonitis and abdominal organs covered with a small amount of fibrinous exudate, and such exudates was also found in their pericardium.

### Brucellosis in pigs

*B. suis* is the only species that causes systemic infection leading to reproductive problems in the swine. Swine can be infected by other *Brucella* species but the infection is invariably self-limiting<sup>[56]</sup>.

Clinical signs of *B. suis* infection in the swine vary considerably, depending on the animal age, previous exposure, and the organ involved<sup>[57]</sup>. Manifestations of swine brucellosis are abortion, birth of weak piglets, infertility, orchitis, epididymitis, spondylitis of especially the lumbar and sacral regions, arthritis, paralysis of hindlimbs, and lameness, but many infected herds may have no signs. There is no pyrexia, and death is rare<sup>[56]</sup>. Abscesses of different sizes frequently occur in organs and tissues<sup>[44]</sup>.

The rate of abortion is higher in sows or gilts exposed to *B. suis* via the genital tract at the time of breeding. Abortions may occur at any time and are influenced more by the time of exposure to the *B. suis* rather than by the stage of gestation<sup>[56]</sup>. Abortions have been observed as early as 17 days following natural insemination by boars disseminating *B. suis* in the semen. Early abortions are usually unnoticed by the owner, and the only evidence of infection is that the sow displays signs of estrus 30 to 45 days after mating. Little or no vaginal discharge is observed in early abortions. Abortions that occur during mid or late stages of gestation are usually associated with females that acquire infection after 35 to 40 days of pregnancy<sup>[57]</sup>. Affected sows rarely have a second abortion, and females infected before sexual maturity hardly ever abort<sup>[44]</sup>. Clinically apparent abnormal vaginal exudate is seldom observed in sows that have uterine infection<sup>[56]</sup>.

Some infected boars do not develop a localized genital infection. However, boars that do develop genital infection seldom recover from it. Infertility and lack of sexual activity

may occur in infected boars and is frequently associated with testicular involvement. More commonly, boars have infections in their accessory genital glands; however this does not necessarily reduce fertility<sup>[56]</sup>. Infection of the genital organs lasts for a shorter period of time in the female than in the male<sup>[44]</sup>.

The disease is more common in adults. Clinical evidence of brucellosis in suckling and weaning piglets is usually absent. Clinical manifestations of arthritis and lameness or spondylitis associated with paralysis of hindlimbs are occasionally observed in any age of swine<sup>[56]</sup>.

#### **Brucellosis in sheep and goats**

Brucellosis in sheep and goats are mainly caused by *B. melitensis* and *B. ovis*. However, other types of *Brucella* can infect sheep and goats. *B. abortus* was isolated from eight sheep and from their offspring over a period of 40 months. *B. suis* was isolated from the semen of a ram<sup>[53, 58]</sup>.

*Brucella* infection produces a chronic disease on sexually mature animals, being the genital tract the main target of the bacteria. *B. melitensis* affects both species, being abortion in females the main clinical sign, whereas *B. ovis* affects only sheep and it is the cause of contagious epididymitis of rams<sup>[59]</sup>. The effect of the disease at flock level is characterized by a general decrease in flock fertility, an increase in lamb/kid mortality with a low weaning percentage, a decrease on milk production and an increased culling of males due to chronic lesion on reproductive organs<sup>[60, 61]</sup>.

#### **Brucellosis in horses**

The infection is caused by *B. abortus* and occasionally *B. suis*. The disease is acquired through contact with infected cattle or swine, ingestion of contaminated food or water and *via* penetration of the skin or mucous membrane. Brucellosis in horses can either remain asymptomatic or it can be associated with clinical disease<sup>[62]</sup>.

Experimental infection with *B. abortus* in horses induced only mild pyrexia. However, granulomatous lesions were observed in lung, liver, testes and metatarsophalangeal synovial membranes. The mares bred normally without abortion or other signs<sup>[63]</sup>. The disease is mainly recognized as an inflammation of the supraspinous and supra-atlantal bursa. These syndromes are known as "fistulous withers" and "poll evil" respectively. The bursal sac becomes distended by clear, viscous, straw-colored exudates and develops a thickened wall. It can rupture, leading to a secondary point of infection. Not all cases of poll evil and fistulous withers are associated with *Brucella*; other bacteria such as *Streptococcus zooepidemicus* may be involved. *Brucella* associated abortions are rare in horses. Other clinical signs reported in horses due *B. abortus* infection are arthritis, intermittent lameness, lethargy and swelling of carpal joint<sup>[64]</sup>.

#### **Brucellosis in dogs**

*Brucella canis* infection is one of the main infectious causes of reproductive disorders in wild and domestic dogs. The greatest prevalence rates probably occur among breeding dogs in commercial kennels where significant reproductive losses can be seen. Up to 75% fewer puppies may be weaned from affected kennels according the hygienic and sanitary conditions<sup>[65]</sup>.

Clinical signs vary from asymptomatic to mild, despite an ongoing systemic infection. Morbidity is high but mortality is low. Bacteremia develops within two to three weeks after

infection; the incubation period to clinical reproductive signs is variable<sup>[66]</sup>.

The classic sign of canine brucellosis is late abortion, which can occur between 30-57 days of gestation, being more common from 45 to 55 days of gestation in about 75% of the cases. Abortions are followed by mucoid, serosanguineous, brownish or gray vaginal discharge that persists for up to six weeks<sup>[66]</sup>. Brucellosis does not change the presentation of estrus and breeding<sup>[66]</sup>. The infected female can produce consecutive abortions and present litters of sick-born pups that die a few hours to more than one month after delivery. Birth of apparently normal offspring who develop the disease later can occur<sup>[67]</sup>. Abortions, premature litters and conception failures are observed in infected kennels<sup>[65, 68]</sup>.

Resorption or early embryonic death within 2 to 3 weeks after breeding can also occur<sup>[66]</sup>. The female is considered infertile since no outward signs of fetal death were seen. Pups are lost as early as 20 days or are carried nearly to term. Infected bitches may deliver a normal litter the next pregnancy or give birth to living, partly autolyzed, stillborn and normal pups that die within hours. The surviving pups are bacteremic for a minimum of several months<sup>[66]</sup>, other congenitally infected pups can be born normal and later develop brucellosis. Some pups have transient fever, leukocytosis or seizures as manifestations of systemic infection. The bitch can abort two to three litters consecutively, continue to be bred and have a normal litter later<sup>[66]</sup>. Clinical signs can occur during subsequent pregnancies in some dogs but not in all of them<sup>[66]</sup>.

*B. canis* targets androgen-dependent organs in the infected dog. The main clinical manifestations in males are severe epididymitis, orchitis and prostatitis. Epididymitis usually begins 5 weeks after infection. Acute onset of inflammation with pain and swelling enables the physical examination detection of orchitis and epididymitis. During the acute phase, the epididymis increases in size, accompanied by pain and presence of serosanguineous fluid in the tunica. Scrotal dermatitis develops from a constant licking by the male leading to edema and dermatitis which usually presents secondary contamination by non-hemolytic staphylococci<sup>[66]</sup>. Dogs with brucellosis may recover spontaneously as soon as one year after infection, but recovery is more common after 2 to 3 years after it, and some dogs remain chronically infected for at least five years<sup>[66]</sup>. Cats are relatively resistant to *Brucella* infections.

#### **In other species**

Bison, elk, reindeer and caribou can be infected with *Brucella spp.*, but their role in transmission of the infections to livestock remains under debate<sup>[3]</sup>.

Most recently, *Brucella spp.* (different from those in land mammals) were isolated from dolphins, seals and an otter. Experimentally, at least one of the marine mammal species can infect and cause abortion in cattle, and marine mammal species have also been isolated from human beings<sup>[2]</sup>.

#### **Brucellosis in humans**

*B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, and marine mammal *Brucella* species are human pathogens<sup>[2, 4]</sup>. In humans, brucellosis can be a serious, debilitating and sometimes chronic disease that may affect a variety of organs<sup>[30]</sup>. Most cases are caused by occupational exposure to infected animals or the ingestion of unpasteurized dairy products<sup>[4, 8]</sup>.

Complications and involvement of internal organs can be diverse, depending on the site of infection, and include encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis [44]. Although unusual, spontaneous abortions have been seen in pregnant women infected with *Brucella* in early stages [49].

### Laboratory diagnosis

Culture from the blood of an animal provides definite proof of brucellosis [69]. Blood may be cultured using the biphasic method of Castaneda which uses both a solid and a liquid medium in the same container [70]. This method circumvents the need for subculturing and is used to limit the risk of laboratory-acquired infections. *Brucella* however, is a slow growing organism and cultures are rarely positive before the fourth day of incubation. Usually cultures become positive between the first and third week, and should be kept for at least 45 days before the culture can be concluded to be negative for *Brucella*. The modern semi-automatic culture systems have somewhat improved the speed of detection but are still too slow to make a fast diagnosis. Another improvement which one might consider is the identification of the organism in positive blood cultures by a modification of the cold ZN staining [71]. Because of the slowness of culture one often moves to serology [72]. Most serological tests rely on the unique antigenic properties of LPS that are shared among the *Brucella* species. The use of LPS as antigen causes cross reactivity with organisms such as *Vibrio* and *Yersinia enterocolitica* that share common features of the LPS [73]. More important in the use and interpretation of LPS based testing is the fact that in endemic areas a large proportion of the population may have developed antibodies due to previous disease or exposure.

The classical Rose Bengal test (RB) is often used as a rapid screening test [74]. RB is based on the agglutination of serum antibodies with a stained whole cell preparation of killed *Brucella*. The sensitivity of RB is very high (>99%) but the specificity can be disappointingly low [75]. As a result, the positive predictive value of the test is low and a positive test result thus requires confirmation by a more specific test. The negative predictive value of RB though is high and a negative test results excludes active brucellosis with a high degree of certainty. To increase the specificity and the positive predictive value of RB the test may be applied to a serial dilution (1:2 through 1:64) of the serum sample. The specificity of the RB increases when higher dilutions agglutinate and titres of 1:8 or 1:16 and above may be regarded as positive. This approach however inevitably results in a lower sensitivity.

For confirmation of RB the Wright or serum agglutination test (SAT) or in more sophisticated equipped laboratories enzyme linked immunosorbent assay (ELISA) may be used [75, 76]. SAT is performed by mixing serial dilutions of serum, usually 1:20 through 1:2,560, with *Brucella* antigen in test tubes or in wells of an ELISA plate. After overnight incubation agglutination is read either by the unaided eye or under a binocular. As guidance agglutination at titres of 1:160 or above is considered of diagnostic value as long as the animals have signs and symptoms of disease [75, 76].

Sometimes SAT is performed in the presence of the reducing agents 2-mercaptoethanol (2-ME) or dithiothreitol (DTT). These reducing agents destroy the agglutinating activity of immunoglobulin M (IgM) leaving IgG intact [72]. The 2-ME or SAT-DTT test is used to increase the specificity of the

reaction by looking at IgG only, which is important in animals with a more persistent infection.

In brucellosis, specific IgM antibodies dominate during the acute phase of the disease [77]. Specific IgG antibodies are present in the serum of animals at later stages of the illness and in the serum of relapsing animals [78]. ELISA is used to discriminate between the presence of specific IgM and IgG antibodies and to roughly access the stage of illness. SAT and the 2-ME test also are used for this purpose but are less accurate [78].

### Treatment

Uncomplicated acute brucellosis almost invariably responds well to appropriate antibiotic treatment [79, 80]. To prevent disease progression and the development of complications, treatment should be start as early as possible also in animals showing signs of spontaneous improvement. The uncomplicated cases should be treated with doxycycline twice a day for 6 weeks plus streptomycin daily for 2 to 3 weeks. Instead of streptomycin rifampicin may be given in combination with doxycycline [81, 82]. 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 [81, 82]. The optimal therapy for brucellosis during pregnancy has not been established [83]. Co-trimoxazole has been used successfully. Alternatively, rifampicin for at least 45 days may be used.

### Control and prevention of brucellosis in animals

Mass vaccination is crucial for the control and eradication of bovine, ovine and caprine brucellosis but other complementary measures that may need consideration include improved farm hygiene, restriction and control of trade and movement of animals, testing of animals and isolation and removal of infected animals. Though the existing vaccine for bovine brucellosis, the *B. abortus* strain 19 (S19), and the vaccine for ovine and caprine brucellosis, the *B. melitensis* strain Rev 1, have some undesirable traits, these have proven to be very useful under most conditions [84-86]. Given the complexity of the epidemiology of brucellosis involving various animal species, the effective control will require a long lasting and carefully controlled and monitored effort. Therefore, preventive measures will be essential to minimize the risk of infection of the human population [87]. Such measures should include improved food hygiene including the pasteurization of milk and protection from infection of high risk groups such as milkers and other people working in the dairy industry. Health education of risk groups through community participation and health education programmes could play an important role to increase the acceptance and use of preventive measures [87-89].

### Conclusion

Brucellosis in animals and humans is worldwide distributed and being considered one of the most important zoonosis. It is a chronic disease and can develop several clinical forms, presenting from reproductive to systemic clinical signs and sometimes being asymptomatic. Affected symptomatic or asymptomatic animals represent important source of infection eliminating the agent through secretions and excretions. Humans are affected by contact with infected animals or through the ingestion of contaminated food. Marine species of

*Brucella* have been recognized lately and there are reports of human infections with these species, demonstrating the wide range and adaptability of these bacteria.

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