Endemic Brucellosis in Indian Animal and Human Populations: A Billion Dollar Issue

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Abstract
Brucellosis is a common and neglected zoonotic disease. It is endemic and still an uncontrolled public health problem in many developing countries including India. Present epidemiological data suggest that brucellosis is causing a major economic problem which burdens up to 3.4 billion US dollars per year. Due to insufficient awareness in public, safe livestock practices, trading the infected animals and economic burden of disease diagnosis and vaccination have led to persistence of brucellosis in the country. Paucity of epidemiological data obtained from previous survey programmes is not convincing enough with the degree of the disease prevalence in the country. Since India is sensitive from both religious and economic points of view, control of the disease has become a sensitive issue. Prevention of brucellosis is dependent on conducting adequate health education awareness and control programmes to address the disease depth of the issue in the country. This review discusses epidemiology, diagnostic methods, prevalence, and vaccines along with recent control measures adopted in Indian Scenario.

Key words: Brucellosis, animal populations, zoonotic disease, small ruminants, insufficient awareness, paucity of epidemiological data, awareness and control programmes

Introduction
Brucellosis is an infectious zoonotic disease caused by Gram negative facultative intracellular bacterial organisms of the genus Brucella. Brucellae belong to α-2 subdivision of proteobacteria. They are Gram-negative, partially acid fast, aerobic, facultative intracellular coccobacilli or short rods. They are oxidase, catalase, nitrate reductase and urease positive. Brucella can infect a wide variety of animal species and human beings. The genus Brucella contains ten recognized species. Based on the host specificity and pathogenicity and host preference, six classical species are identified (1). They are Brucella abortus, Brucella melitensis, Brucella suis, Brucella ovis, Brucella canis and Brucella neotame (2, 3). Recently, four new Brucella species are identified. They are Brucella pinnipedalis isolated from seals and Brucella ceti isolated from cetaceans (4), Brucella microti isolated from common voles (5), soil (6) and foxes (7) and Brucella inopinata isolated from breast implant (8). Worldwide the main pathogenic species are B. abortus responsible for bovine brucellosis, B. melitensis that causes caprine and ovine brucellosis and B. suis associated with swine brucellosis. These three species mainly cause abortions in animals. B. abortus preferentially infects cattle, B. melitensis sheep.
and goats, *B. suis* pigs and *B. canis* dogs. Above species infect humans with *B. melitensis* being the most common.

Brucellosis is a dreadful and contagious disease of animals and characterized by abortion in females and to a lesser extent orchitis and infection of accessory sex glands in males and infertility in both the sexes. It has zoonotic importance in terms of its transmissibility to human beings. When brucellosis is identified in a herd, flock, region or country, international veterinary regulations impose restrictions on animal movement and trade, which results in huge economic loss. In order to control and eradicate brucellosis in cattle, small ruminants and pigs, many control programmes have been initiated and implemented worldwide (9). *B. ovis* and *B. canis* are responsible for ram epididymitis and canine brucellosis, respectively. In the case of *B. neotomae*, only strains isolated from desert wood rat (*Neotoma lepida*) in North America have been reported.

**Brucellosis and epidemiology**

Brucellosis is found worldwide, however it has been eradicated from many countries, but still it is the most serious problem in developing countries including India. The disease has considerable impact on animal and human health as well as socioeconomic impacts especially in rural areas where income relies largely on livestock breeding and dairy products. The rates of infection may vary from one country to another and between regions within the country. Brucellosis is widely prevalent throughout India among the bovine population both in farm and village animals causing economic losses up to 3.4 billion US dollars (10). In India, prevalence and disease spread is still increasing, though advances in diagnosis, therapy and vaccines have been made available. Serological survey of brucellosis was performed in 23 states of India. A total of 30,437 bovine samples were screened with Rose Bengal Plate Agglutination Test (RBPT) and Serum Tube Agglutination Test (SAT), which revealed 1.9% prevalence in cattle and 1.8% in

<table>
<thead>
<tr>
<th>State</th>
<th>Species</th>
<th>Prevalence</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Rajasthan</td>
<td>Goats, Humans</td>
<td>11.45%, 2.97%</td>
<td>Kapoor <em>et al.</em>, 1985 (12)</td>
</tr>
<tr>
<td>Nagaland</td>
<td>Over all</td>
<td>11% to 34%</td>
<td>Rajkhowa <em>et al.</em>, 2005 (13)</td>
</tr>
<tr>
<td>Punjab</td>
<td>Buffaloes</td>
<td>13.4%</td>
<td>Dhand <em>et al.</em>, 2005 (14)</td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>Cattle</td>
<td>9.96% to 20.35%</td>
<td>Sulima, 2009 (15)</td>
</tr>
<tr>
<td>Rajasthan and Bihar</td>
<td>Over all</td>
<td>8.58%</td>
<td>Singh <em>et al.</em>, 2007 (16)</td>
</tr>
<tr>
<td>Arunachal Pradesh</td>
<td>Sheep</td>
<td>7.08%</td>
<td>Shakuntala <em>et al.</em>, 2016 (17)</td>
</tr>
<tr>
<td>Meghalaya</td>
<td>Over all</td>
<td>2.8 to 5.6%</td>
<td>Shakuntala <em>et al.</em>, 2016 (17)</td>
</tr>
<tr>
<td>Gujarat</td>
<td>Over all</td>
<td>11.90% to 33.70%</td>
<td>Patel, 2014 (18)</td>
</tr>
<tr>
<td>Karnataka</td>
<td>Over all</td>
<td>45.80%</td>
<td>Jagapur, 2013 (19)</td>
</tr>
<tr>
<td>Uttar Pradesh</td>
<td>Over all</td>
<td>22.39%</td>
<td>Jagapur, 2013 (19)</td>
</tr>
<tr>
<td>Uttarakhand</td>
<td>Over all</td>
<td>8.57%</td>
<td>Jagapur, 2013 (19)</td>
</tr>
</tbody>
</table>
buffaloes (11). Seroprevalence of brucellosis in different states of India from previous reports was mentioned in the table 1. From the table, it is evident that prevalence reports were mainly concentrated on live stock species. Seroprevalence from rest of the states is not available at present.

Animal brucellosis: Brucellosis causes economic losses due to abortions, premature births, and decreased milk production in livestock. Further, repeated breeding also may cause temporary or permanent infertility in livestock. 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 across different states (20). Free grazing and mixing with flocks of sheep and goats also contribute to wide disease spread of disease in animals, resulting in an outbreak of brucellosis. Clinical signs of the disease in animals include abortions, retained placenta, orchitis, epididymitis, rarely arthritis, with excretion of organisms in milk and uterine discharges. Diagnosis of disease depends on the isolation of the organism from milk, blood, abortion materials, udder secretions and tissues. Presumptive diagnosis can be carried out by assessing serological responses to Brucella antigens (21). All Brucella species may also infect wild life species. Classical species have been isolated from a great variety of wild species such as bison, elk, wild boar, fox, hare, feral swine, African buffalo, reindeer and caribou (22). In order to implement appropriate control methods to address wild life brucellosis, it is crucial to distinguish between spill over infection contracted from domestic animals and a sustainable infection (22). In the latter case, the concern of the livestock industry is to prevent the re-introduction of the infection in livestock (spill-back), particularly in regions or states that are “officially brucellosis-free”. If the status of “officially brucellosis-free” is lost, domestic animals must be tested prior to being traded, which imposes huge costs.

Human brucellosis: Brucellosis is a significant zoonoses that causes veterinary and public health problems in India. In India, 80% of the population live in approximately 575,000 villages and thousands of small towns; have close contact with domestic or wild animal population owing to their occupation. Hence, human population stands at a greater risk of acquiring zoonotic diseases including brucellosis (23). Human brucellosis is predominantly transmitted through animal contact and also by consuming infected milk and meat products. Worldwide, reported incidence of human brucellosis in endemic disease areas varies widely, from <0.01 to >200 per 100,000 population. For example, Egypt, the Islamic Republic of Iran, Jordan, Oman, Saudi Arabia and Syrian Arab Republic reported a combined annual total of more than 90,000 cases of human brucellosis in 1990 (24). The low incidence reported in known brucellosis-endemic areas may reflect the absence or the low levels of surveillance and reporting (25). However in India, the true incidence of human brucellosis is unknown.

B. abortus, B. melitensis, B. suis and B. canis are the species that mainly infect humans. Consumption of undercooked traditional delicacies has been implicated in human brucellosis. Other route of infection for persons working in slaughter houses, laboratories and veterinarians is through skin wounds or by accidental ingestion. Hunters may be infected by skin abrasions or by accidental ingestion of organisms of animals that they have killed (26, 27). Inhalation is often responsible for a significant percentage of cases in abattoir employees (28). Laboratory acquired Brucella infection could be through accidental inhalation of aerosols and mucosal or skin contacts. Brucellosis is a major health hazard for the laboratory workers handling the cultures of the virulent or attenuated 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 (Bouza et al 2005 (29); Centre for Disease Control and Prevention [CDC] 2008 (30)). The presence of brucellosis in wild animals, with a potential for continuous transfer to domestic animals and from them to humans is another
epidemiological issue (31). *B. melitensis* has been identified in most of the recorded cases in humans. However, *B. abortus* and *B. suis* also cause substantial morbidity in the countries where domestic animals have persistent infection. *B. canis* is rarely seen in humans and *B. ovis*, and *B. neotamae* have not been identified in humans. Clinical signs and symptoms of human infection include continued or intermittent fever, chills, irregular fever of variable duration, with headache, profuse sweating, weakness and weight loss. Although brucellosis has been or close to being eradicated in developed countries, it is still a major public and animal health problem particularly in developing countries where livestock are major sources of food and income. Livestock expansion with uncontrolled transport, lack of veterinary support and vaccines favouring the disease are spreading. Many factors are associated with the current approaches of disease control, eradication or prevention in the country such as level of infection, reliability of diagnostic tests, surveillance, monitoring and effective vaccination programmes.

**Brucellosis diagnosis** : Diagnosis of brucellosis based on clinical signs is difficult, because they are also commonly observed in other diseases. The most important clinical feature of brucellosis is abortion in their first gestation. Usually, females abort only once and remain infected throughout their life time. So, clinical diagnosis of the disease in animals cannot be fixed on the basis of abortion, since many pathogens may cause abortions in animals. Clinicians practicing in endemic areas must be aware of the disease and develop a high degree of clinical suspension based on the history and epidemiological data. Otherwise, the disease may be misdiagnosed or diagnosis may be delayed, due to its deceptive nature. Therefore, laboratory testing is essential. Brucellosis diagnosis involves direct testing such as isolation and identification of *Brucella* species. Indirect testing includes detection of antibodies in blood and milk specific to *Brucella* antigens. The choice of testing depends upon the prevalence of the disease and epidemiological status of disease suspected animals in a country or region. Isolation of organisms and detection of *Brucella* species DNA are the methods that allow certainty of diagnosis.

**Culture of Brucella species in the laboratory** : Definitive diagnosis of brucellosis is carried out by isolation and culture of organism from the clinical samples. Laboratory diagnosis of disease by culture method is slow due to slow growth of *Brucella* in culture media (32, 33). Bacteriological method includes culturing of samples such as aborted foetal stomach contents, milk, blood, lymph nodes and vaginal discharges from suspected cases for isolation and identification of the infecting *Brucella* organisms (34, 35). The isolated organisms are further tested using molecular based tests. This sequence of confirmatory procedures is referred to as the “gold standard” method for identifying *Brucella* species (36).

**Molecular detection of brucellosis** : Molecular methods such as polymerase chain reaction (PCR) have been recently included in disease diagnosis. The advantages of PCR are numerous. Independent of the disease stage, it is more sensitive than blood culture and more specific than serological methods (37). Genus specific PCR assays are generally adequate for the molecular diagnosis of human brucellosis (38). Molecular assays targeting the *IS711* insertion element, which is found in multiple copies within *Brucella* chromosomes, also improve analytical sensitivity (39). The *bcsp31* gene, coding for a 31-kDa immunogenic outer membrane protein conserved among all *Brucella* spp. is the most common molecular target in clinical applications (40). Such a genus-specific PCR can help to avoid false-negative results in patients infected with unusual species and biovars.

**Serological methods of brucellosis detection** : Several serological methods have been tried and few of them have lasting effects. Rose Bengal Plate Agglutination Test (RBPT), Serum Tube Agglutination Test (SAT), Enzyme Linked Immunosorbent Assay (ELISA) are the most

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common tests practiced in the laboratories now. Recently, Lateral Flow Assays (LFA) are also widely used in laboratories and in the field. According to OIE, Complement Fixation Test (CFT) and Competitive ELISA (cELISA) are the gold standard serological assays being used for disease diagnosis. Other important assays used for diagnosis include coombs agglutination test, radio immune assay, and fluorescent polarization assay.

**Rose Bengal Plate Agglutination Test for brucellosis diagnosis**: RBPT is a widely used diagnosis agglutination test. It is rapid and can be performed within 4 minutes on glass slide or plate with the help of an acidic-buffered antigen (pH 3.65 ± 0.05). This test has been introduced as screening test in the field because of its rapidity and simplicity. OIE considers agglutination tests are “prescribed tests for trade” (OIE 2009).

**Serum Agglutination Test for diagnosis**: SAT is the most popular and is recently being used worldwide as a diagnosis test. SAT measures the total quantity of agglutinating antibodies (IgM and IgG), and the quantity of specific IgG is determined by 2-mercaptoethanol (2ME). SAT titers above 1:160 are considered for diagnostic purposes in conjunction with a compatible clinical presentation. In endemic areas, a titre of 1:320 as cutoff may make the test more specific. The type of antibody is important, as IgG antibodies are considered a better indicator of active infection and the rapid fall in the level of IgG antibodies is said to be prognostic of successful therapy. Studies by researchers (41, 42) have shown persistence of various levels of SAT antibodies in many clinically cured patients.

**Enzyme Linked Immunosorbent Assay test for diagnosis**: ELISA are divided into two categories. One is indirect ELISA (iELISA) and another competitive ELISA (cELISA). ELISA is an effective method for diagnosing acute and chronic brucellosis and for detecting antibodies in CSF of patients with neurobrucellosis. The ELISA is as sensitive as Radio Immuno Assay (RIA) (43). In iELISA, mostly purified smooth LPS is used as an antigen but good deal of variation exists in the anti-bovine IgG conjugate (44). iELISA is highly sensitive but vulnerable to non-specific reactions with *Yersinia enterocolitica* O:9. In cELISA, monoclonal antibodies developed against specific epitope that are not shared with LPS of *Yersinia* are used to increase the specificity of the assay (45, 46).

**Complement Fixation Test (CFT) for brucellosis diagnosis**: CFT allows detection of anti-*Brucella* antibodies that are able to activate complement. Cattle immunoglobulins (Ig) that can activate bovine complement are the IgG and the IgM. According to available literature, this test is not highly sensitive but exhibits an excellent specificity (47, 48). Since the test is difficult to standardize, it is progressively being replaced by ELISAs (OIE 2009).

**Fluorescence Polarization Assay (FPA)**: During FPA test, serum samples are incubated with *Brucella* specific antigen labeled with fluorescein isothiocyanate. Large fluorescent complexes are formed in the presence of antibodies against *Brucella* species. In negative samples, antibodies do not form a complex and spin quickly, therefore cause greater depolarization of light. In the positive samples, antibodies specific to *Brucella* antigen form complexes and cause less depolarization of light than do in negative samples. Thus, the disease is detected based on light polarization.

**Lateral flow assays (LFA) for diagnosing brucellosis**: LFA is also called as rapid immuno chromatographic assay. *Brucella* IgG and IgM lateral flow assays (49) and protein-G based lateral flow assays (50) have been found to be rapid and simple with high sensitivity and specificity. These tests are simple, rapid and can be performed easily in point of care areas and health care centers as field tests. Thus, LFA has an edge over other diagnostic tests.

**Spectrum of brucellosis disease**
Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India, has launched a “network project on brucellosis”. The
mission of the network is to develop simple, rapid and convenient diagnostic kits like lateral flow assay (LFA) rapid detection kits (Figure 1) and indirect ELISA (iELISA) kits (Figure 2) and validate them at the National Reference Laboratories. These kits are currently being used in the field to understand the prevalence of brucellosis in a large spectrum. In the present study, a total of 14,343 samples were collected randomly from various parts of the country, including domestic animals, wild animals and humans involved in animal practices (Table 2). The data suggest that over all the disease seroprevalence is 15.15%. All these samples were categorized into three types based on the places of testing. They are Gosalas, unorganized village farms and organized dairy farms. Out of this data, screening of Gosalas, unorganized village farms, and organized dairy farms showed that the prevalence of the disease is 30.81%, 10.09% and 11.62% respectively.

In Gosalas, shelter less and unwanted cows gather from different places and become a reservoir for brucellosis and other infectious diseases. Due to insufficient awareness, any one of the cows from infected area/region with brucellosis easily transmits the disease to the other healthy animals present in Gosalas and neighbouring areas. In our study, screening of 4,085 animals from 10 Gosalas was carried out by LFA and iELISA tests which revealed that prevalence ranges from 10.24% to 30.81%. Screening of 4,695 animals for disease prevalence in organized dairy farms showed 5.24% to 17.82%. Contrarily, in unorganized village farms, the disease prevalence is less and ranged from 0.1% to 10.09%. Shome et al. (51) reported 0.58% to 20.17% in individual animal seroprevalence in organized farms. Examination of 4,580 animals by RBPT and ELISA tests from 119 dairy farms revealed high over all prevalence (65.54%) of the disease in herds than in individual animals in Punjab (34.15%) and Haryana (22.34%) (52). In unorganized farms, high prevalence (14.14%) was recorded in comparison with organized farms (3.23%) by Lone et al. (53). In contrast, other reports (54, 55) showed higher prevalence of brucellosis among the organized farms compared to the rural unorganized farms. Radostits et al. (56) indicated that the prevalence of the disease depends upon diverse factors like management, housing, animal population density, size of farm, type of herd (self-raised or purchased from different sources), sanitary conditions and the method of disposal of infected animals.

Brucellosis and its significance in public health

Human brucellosis has serious public health consequences in endemic areas (57). In humans it represents a major public health hazard, which affects social and economic development in various countries. Animal health workers, butchers, farmers, and those who habitually consume raw milk and come in contact with animals are at high risk for catching brucellosis (58). Veterinarians and laboratory persons who work with Brucella cultures are also at high risk of getting infected.

Treatment, vaccination and control programmes

Brucellae are inaccessible to antibiotics as they are facultative, intracellular pathogens. Many antimicrobials are active against Brucella species; however, clinical efficacy does not always correlate with in vitro susceptibility (59). The treatment recommended by the World Health Organization for acute brucellosis in adults is rifampicin 600 to 900 mg and doxycycline 100 mg

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Table 2. Test results of LFA and iELISA for brucellosis testing with field collected samples.

<table>
<thead>
<tr>
<th>Species</th>
<th>No. of samples tested</th>
<th>No. of positive samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows and Bulls</td>
<td>4635</td>
<td>1264 (27%)</td>
</tr>
<tr>
<td>Buffaloes</td>
<td>4977</td>
<td>547 (10.9%)</td>
</tr>
<tr>
<td>Sheep and Goats</td>
<td>571</td>
<td>14 (2.4%)</td>
</tr>
<tr>
<td>Pigs</td>
<td>113</td>
<td>2 (1.8%)</td>
</tr>
<tr>
<td>Dogs</td>
<td>223</td>
<td>4 (1.8%)</td>
</tr>
<tr>
<td>Elephants</td>
<td>29</td>
<td>6 (20.6%)</td>
</tr>
<tr>
<td>Camels</td>
<td>55</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Humans</td>
<td>516</td>
<td>13 (2.5%)</td>
</tr>
<tr>
<td>Transterrestrial</td>
<td>3224</td>
<td>317 (9.8%)</td>
</tr>
<tr>
<td>Total samples</td>
<td>14,343</td>
<td>2,173 (15.15%)</td>
</tr>
</tbody>
</table>

Twice daily for a minimum of six weeks (FAO/WHO 1986). Some still claim that a combination of intramuscular streptomycin (1 g/day for 2-3 weeks) with an oral tetracycline (2 g/day for 6 weeks) gives fewer relapses (60, 42). Trimethoprim-sulfamethoxazole (TMP/SMX) is a popular drug in many areas, used in triple regimens. Various combinations that incorporate ciprofloxacin and ofloxacin have been tried clinically, yielding similar efficacy to that of the classic regimens (61). For neurobrucellosis, combination therapy with two or three drugs - doxycycline, rifampicin, and TMP/SMX that penetrate central nervous system is recommended (62). In case of animals, the amount of drugs needed for the exercise outweigh the economic value of an animal. Most farmers do not have the capacity to continue with the treatment because it is time consuming and drugs very expensive. Therefore, vaccination of young animals has proved to be the best preventive measure.

There are a number of approaches in the control of brucellosis and eradication programmes which include vaccination of animals, surveillance, testing, quarantine and culling (63, 64). Animal vaccination, diagnosis (serological and molecular methods) and culling programmes have been implemented in many countries like USA, UK and Canada and freed the areas from disease for some
years, although incidental cases are reported due to relaxation of the above mentioned programmes. Other factor associated with high prevalence is increasing exchange of animals harbouring *Brucella* organisms (65, 66). Animal vaccination in endemic areas has been the most effective control method. An attenuated vaccine strain that induces a T-cell mediated immune response grants a more improved immunity than the killed vaccines (67). In many countries, S19 is a widely used vaccine for the control of the disease. Recently, RB51 developed from rough strain of *B. abortus* has been introduced into the market. However, S19 is still the most effective vaccine used to control brucellosis. The attenuated strain is a live vaccine that ignites the immune response of the vaccinated animal to resist *Brucella* infection by producing antibodies against the attacking organisms and getting rid of the dead organisms by phagocytes. The antibodies produced against the disease disappear from the systemic circulation in few months although lifelong immunity has been suggested so that the animal retains the resistance to disease for years (67). In India, S19 is produced in large scale and the most widely used. Testing and culling may help in screening and confirming suspected cases and at the same time getting rid of *Brucella* positive animals. While in some cases, cross reactions give false positives resulting into culling wrong animals. Therefore, caution must be exercised while testing for the disease diagnosis and subsequent culling.

How to address brucellosis in India

In India, lack of awareness, cost effectiveness of vaccination and lack of proper diagnosis of animals during trading, economic burden of screening the disease and vaccination of animals have led the persistence of brucellosis. Insufficient preventive measures and lack of awareness in rural areas as well as uncontrolled selling/transport of animals in open borders are resulting in high prevalence. Further, high prevalence is also due to armed conflicts and political instability in the country and previously unsuccessful eradication programmes in the country.

Since India is sensitive from both religious and economic points of view, culling the infected animals is not possible. This can be accounted for organized farms, unorganized village farms and Gosalas too. Prevention of human brucellosis should be focused mainly on the elimination of infection in animals and humans along with hygiene, vaccination and effective heating and pasteurization of dairy products. Although India has a policy for the control of brucellosis in dairy cattle, the present focus is very much towards the curative services rather than preventive. Veterinarians and other health care workers should take precautionary measures such as wearing protective clothes, gloves and masks while handling the still births, conception materials and cultures. Such measures can reduce the occupational risk of acquiring brucellosis (68). In general population, avoidance of unpasteurized dairy products, raw meat consumption can certainly prevent infection.

Conclusions

In India, brucellosis Prevalence is more in Gosalas, organized dairy farms according to the available data. As brucellosis transmitted from small ruminants poses a significant health risk factor, more efforts should be required to diagnose and control brucellosis in sheep and goats also. Organizing adequate awareness and disease control programmes on public health education on brucellosis, and risk factors can prevent rapid spreading of brucellosis. Quarantine of suspected or animals in transit must be screened for the disease in order to prevent transmission of brucellosis from region to region. Since animal brucellosis treatment 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 India, lack of awareness and limited availability of vaccines are the main causes for persistence of brucellosis. A paradigm shift from the current biomedical model to a socio-cultural model is imperative for the control and elimination of brucellosis in India. Brucellosis is a serious public
health challenge having socio-economic problems and an unaccounted financial burden which needs joint efforts, promotion of intersectoral action, regional and international cooperation, as well as technical and financial support (69).

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