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BOVINE BRUCELLOSIS: AN EMERGING THREAT TO DAIRY SECTOR IN INDIA

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ABSTRACT

Brucellosis is highly contagious bacterial zoonotic disease and one of world's major zoonoses with veterinary and public health importance. It has serious economic concerns in many parts of the world. Effective national programmes for its prevention and control needs to be developed and implemented. Vaccination in animals is required to be clubbed with other routine vaccination programmes of state animal husbandry departments. Governments and institutes should realize underlying dangers by this disease and promote research focused on development of effective treatment molecules and preventive methodologies.

Key words: Abortion, Bovine, Brucella, Brucellosis, Cattle, Infertility, Reproduction

India has the largest livestock population in the world and is world's largest milk producer. According to the 19thLivestock Census (2012) country has about 190 million cattle, 108 million buffaloes, 135 million goats, 65 million sheep and 10 million pigs. It accounted for about 57 percent of buffalo and 14 percent of the cattle population of the world. In India, dairy farming is becoming more intensive and organized, and farmers are adopting it for diversification by each passing day due to continuous shrinkage of land holdings accompanied by various other socio-economic factors. Dairy animal population is increasing consistently and Livestock Census 2007 pegged the combined cattle and buffalo population at 303 million (198.3 million cattle and 104.7 million buffalo; USDA Foreign Agricultural Service Report, GAIN Report Number: dated: Sep.22, 2010).Reproductive proficiency is one of major consideration in any dairy or livestock production enterprise for sustainable profitability. Apart from mastitis, various reproductive disorders viz; endometritis, repeat breeding,abortions and retained placenta etc. are the main impediments to profitability in organized dairy farms in India.

The causes of infertility and abortions are many, both specific and nonspecific. The specific agents contribute directly to manifestation of infertility leading to reproduction and production losses and role of many such etiologicalagents viz. *Brucella* spp., *Campylobacter* spp., *Chlamydiapsittaci*, *Listeria monocytogenes, Coxiella burnetii*, *Mycoplasma bovigenitalium, Salmonella* spp. and *Leptospira* spp has been well established (Radostitis *et al.,* 2007).

Among various reproductive infections of domestic animals, brucellosis is highly contagious bacterial zoonotic disease affecting both livestock and humans.It is considered to be true zoonoses (basically transmitted from animals to humans) and is one of world's major zoonoses which are of veterinary, human, public health importance and of much economic concern in many parts of the world. It has been reported invariably all domestic animals, humans and also scarcely reported in wild animals and marine (Singh *et al.*, 2015).

Brucellosis in livestock and humans is caused by various species of the genus *Brucella*, which are facultative, intracellular, gram negative coccobacilli that lack capsules, flagella and endospore. Brucellosis is usually caused by *B. abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs. Some species of *Brucella* contain biovars; nine for *B. abortus,* three for *B. melitensis* and five biovars for *B. suis*. In humans, it is transmitted from infected domestic animals and the causal bacteria are *B. abortus*, *B. melitensis*, *B. suis* (biovars 1-4) and rarely *B. canis* or marine mammal *Brucella*. In general, *B. melitensis* is the dominant causative agent of brucellosis in humans and is more infectious than *B. abortus*, although disease caused by *B. abortus* is indistinguishable to that of *B. melitensis* (Dokuzoguz *et al.*, 2005). Live vaccines for *B. abortus, B. melitensis and* M- strain of *B. canis* are also pathogenic for humans while *B. ovis*, *B. neotomae* and *B. suis* biovar 5 have not been reported to cause human disease.

Cattle are susceptible once they have reached sexual maturity. Infection persists in the host once infected. The disease very often spreads from animal to animal in a herd by several modes of transfer, mainly by contact with infected discharges from an aborted dam and its fetus. Infection occurs by ingestion, penetration of the intact skin, conjunctiva and contamination of the udder during milking. The adult cattle may abort during late pregnancy. However, no obvious clinical signs/symptoms appear in brucellosis infected immature or non-pregnant animals. In cattle, *B. abortus* causes abortions, stillbirths and weak calves; abortions may occur during mostly during the late pregnancy (last trimester), associated with retention of placenta and reduced milk yield. Higher incidence of retention of placenta following abortions in such animals frequently leads to pyometra and death may occur as a result of acute metritis (Radostitis *et al.,* 2007). After the first abortion, subsequent pregnancies are generally normal; however, female bovines may become carrier and shed bacteria in milk and uterine discharges. Brucellosis causes orchitis, epididymitis, seminal vesiculitis and sometimes testicular abscesses in affected bulls. Occasionally, hygromas particularly of leg joints develop in males and is common symptom observed in some

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tropical countries. Infertility occurs occasionally in both sexes due to metritis or orchitis/epididymitis. Affected animals may remain sterile for the entire life. Systemic signs usually do not occur in uncomplicated infections and deaths are rare except in the fetus or newborn.

Although brucellosis is mainly implicated for inducing abortions in livestock, it significantly causes reproductive losses. Considering the losses (approx. Rs 1.25 lakhs per abortion) due to brucellosis in terms of decreased milk production, abortions (loss of calf), reduced conception rates, weak offspring, reduced calf crop, weight loss, infertility, poor semen quality and lameness, it is one of the most serious diseases of livestock with great economic and public health importance and is a major impediment for the trade and export of animals, semen, meat and milk products (Gul and Khan, 2007).

Zoonotic aspects and public health importance

Brucellosis is an important but neglected emerging endemic zoonotic communicable disease in India. Farmer, veterinarians, shepherds, abattoir workers, milk plant and meat product plant personnel, hunters and livestock producers are always at high risk as an occupational hazard. Brucellosis is also one of the most easily acquired laboratory infections. It has been recognized as one of common laboratory transmitted infections particularly occurring in clinical, production and research laboratories (Bouza *et al.*, 2005; CDC, 2008).

In India, 742 million rural people residing in 638,588 villages and thousands of small towns remain in close contact with domestic and wild animals because of their dependence on animals for draught power, agriculture operations, milk and other animal products which make asizable Indian population at a greater risk of acquiring a zoonotic disease including brucellosis. The environment and the circumstances like poor sanitation and hygiene, poor socio-economic status, illiteracy, lack of awareness and poor health infrastructure and services are conducive for the spread of this disease in rural households than those in urban areas in India.

Brucella spp. is of interest as they are categorized as biological agents due to their high contagiousness and impact on human and animal health. The zoonotic pathogens *B. abortus, B. melitensis,* and *B. suis* were designated as agents of Category 'B' by the Centre for Disease Control (CDC, Atlanta, USA). Another issue of concern is the use of *Brucellae* as a biological weapon. Although there is no reported case of bio-terrorism using *Brucella spp.* (Shareef, 2006), nevertheless, *Brucellae* are not difficult to grow and disperse (the American military weaponized *Brucella suis* in 1954).

Bovine brucellosis, the most economically devastating reproductive disease in male and female is rapidly growing causing concern for the farmer, veterinarians, livestock supervisors and finally farmer's risk of his own family health. The transmission to humans may result in prolonged illness and long-term sequelae (Yagupsky and Baron, 2005).

Brucellosis is a serious public health problem in many developing countries particularly, in regions of high endemicity such as Africa, the Mediterranean, the Middle East, parts of Asia and Latin America. Though brucellosis has been eradicated in many developed countries, it remains largely an uncontrolled and neglected disease in India. The main impact is economic; deaths are rare except in the fetus and neonate. The treatment of animal brucellosis is very expensive and often unsuccessful and compensation for slaughter of infected animals is not available.

Incidence/Prevalence of brucellosis

Although brucellosis and its mode of transmission were discovered over 100 years ago, it remains a worldwide problem, predominantly so in developing countries. Prevalence of bovine brucellosis and transmission of infection to the human population has been significantly decreased following effective vaccination-based control and prevention programmes with increased sero-surveillance and monitoring in many developed countries. However, it remains widely distributed throughout the developing world and is considered to be serious problem in at least 86 courtiers including India. Brucellosis is an uncontrolled problem in high endemic regions such as the Mediterranean, Middle East, Africa, Latin America and western Asia (Corbel, 1997; Refai, 2002) of the developing world.

Bovine brucellosis is widespread in India and it was first recognized in 1942. The prevalence of brucellosis in different animal species varied widely and appears to be on the increase in recent times, perhaps due to increased trade of animals, meat and dairy products accompanied with intensified farming practices. Verma *et al.* (2000) reported brucellosis ranging from 1.81 to 2.45 % in aborted and infertile small as well as large ruminants in Himachal Pradesh. The long term serological studies carried out by Renukaradhya *et al*. (2002) indicated 5 % of cattle and 3% of buffaloes to be infected with brucellosis in India. They further observed that cumulative incidence of this disease was 7.9 % in sheep and 2.2 % in goats in 10 of the 28 states. In a recent study, an overall sero-prevalance of bovine brucellosis was recorded to be 22.18% (132/595) and 13.78% (82/595) by ELISA and RBPT, respectively in organized dairy farms with a history of abortion in different geographical zones of India (Trangadia *et al.*, 2010). Survey of the brucellosis from 1948 to 2009 indicates its prevalence in Indian cattle and buffalo to be ranging from 1.81 to 20.7% of the tested samples (Kumar, 2010). Serosurveillance studies conducted (using ELISA test) in PVK, LUVAS, Uchani in Karnal during 2009-2010 indicated that 73% cows and 80% of buffaloes having history of recent abortions and surprisingly 33% cows and 64% buffaloes from organized dairy farms with no history of abortions had brucellosis

(Annual Report of Veterinary Unit, Karnal, 2009-10). The survey of ICAR's PD-ADMAS Bangalore also indicated a rising trend in the prevalence of bovine brucellosis.

Factors responsible for spread of the disease

The factors responsible for the spread of *Brucella* organisms include sanitary, socioeconomic, animal husbandry, farming, political etc. In India animals roamed free for grazing and combined with different prevalent livestock get ample opportunity for intermixing through traditional animal husbandry systems which favoured the transmission of the infection. The changing and fast growing dairy industry in India has resulted in intensified trade and animal movements, which provide a new and increased risk in spreading the infection (Renukaradhya *et al.*, 2002). This uncontrolled animal transportation through "open" borders increases the risk for the spread of this disease from endemic to non- endemic areas.

Brucella spp can survive for long period in aborted fetuses, soil, dairy products, meat, dust, dung manure, hay, water, slurry, equipments, wool and clothes. Moreover, in conditions of high humidity and low temperatures (no sunlight), organisms remain viable for several months. *Brucella* can withstand drying, particularly when organic material is present, and can survive in dust and soil. Survival is longer when the temperature is low, particularly when it is below freezing. Since *Brucella* organism survives in liquid nitrogen with semen straw and can spread rapidly through frozen semen being used for artificial insemination. The preponderance of natural bull service in rural areas especially in buffaloes is yet another important factor in the maintenance and spread of infection. As the infectious dose is very low (particularly of *B. melitensis,*10 organisms only), it is an high occupational risk for farmers, veterinarians, abattoir workers, laboratory personnel, farm workers and others who work with animals and consume their products (Smits and Cutler, 2004). Crushing of umbilical cord of newborn kids or lambs with teeth by shepherds and skinning of stillborn lambs and kids and aborted bovine fetuses, which may have heavy *Brucella* spp. load, also present high occupational risk of brucellosis.

Insufficient preventive measures and lack of adequate control programs further add to the spread of brucellosis. Although bacterial load in muscle tissues of affected animals is low, but consumption of customary/traditional raw or undercooked delicacies containing infective tissues or body secretions (liver) also contribute to human infections. Improper disposal (no burying or destruction) of abortion materials containing high *Brucella* load contribute to the spread of the infection. It is important to realize that open water sources such as ponds and wells become a source of infection by contamination with any *Brucella* infected material or wastewater from brucellosis infected farms.

Human brucellosis was once thought to be predominantly transmitted through animal contact. However, it is now being increasingly realized that animal products such as milk and meat products are frequently the source of disease transmission (Kochar *et al.*, 2007). Dairy products prepared from unpasteurized milk such as soft cheese, yoghurts and ice-cream have high concentration of *Brucella a*nd consumption of these is an important source of transmission of this infection. 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; CDC, 2008) as accidental ingestion, inhalation and mucosal or abraded skin contact is a major health hazard for the laboratory workers handling cultures of virulent or avirulent attenuated strains. The increase in business and leisure travel to brucellosis-endemic countries has led to importation of the disease into non-endemic areas (Corbel, 2006).

Preventive and control measures

Learning lessons from brucellosis free countries, countries like India where brucellosis is widespread in cattle and buffaloes measures to prevent the spread of this disease including statutory mass vaccination of livestock, constant monitoring and sero-surveillance of livestock population, improved animal husbandry practices etc. are required. The other methods of prevention are health education aimed at increasing awareness of risks, encouraging health promotion, disease prevention, intervention measures, implementation of local, regional and international standards related to food safety, enhanced regulations on the trading of animals and animal products at national and international levels. Animal owners should be taught about the importance of vaccination of their animals. At present mass vaccination and regular follow-up at village level, milk sample testing and maintaining vaccinated herds in villages and dairy farms and preventing entry of any infected animal and material are the effective ways to bring down the incidence of brucellosis in countries like India where test and segregate, culling/slaughter of infected animals is not practically fool proof and economically viable and for various socio-economic reasons. With ever increasing number of bull mother farms in the central and state setups and larger no of A.I. centers, accredited testing labs needed to be opened in more numbers.

In India, 742 million people live in rural areas in 638,588 villages and thousands of small towns. About five million households in the country are engaged in the rearing of small ruminants (sheep and goats) and other allied activities. Hence, bovines and humans stand at a greater risk of acquiring zoonotic diseases including brucellosis from sheep and goat population also. The disease has an added importance in countries like India, where conditions are conducive for wide-spread human infection on account of unhygienic conditions and poverty. As brucellosis is transmitted from small ruminants, therefore, efforts are required to control brucellosis in goats and sheep.

While effective control measures still need to be implemented, doctors and other health workers may help patients and risk groups to prevent brucellosis by teaching them essential methods to prevent exposure to the pathogen such as boiling of milk and to avoid the consumption of dairy products prepared from unpasteurized milk. Prevention of human brucellosis depends on control of the disease in domestic livestock. Also, control programme for human brucellosis would depend to a large extent on public health education about the risk factors involved in spread of disease, good administrative arrangement and ensuring the maximum co-operation between health and veterinary authorities. It is important to include brucellosis in public health education and to come out of a reductionist approach focused only from a veterinary perspective. At present, there is no national programme focusing on brucellosis. Little attention is given to curative services and livestock vaccination. Focus on prevention, health education and safe livestock practices awareness is also lacking. Most of the research work carried is laboratory-based, focusing on clinical aspects while ignoring the socio-cultural and other conditions responsible for the spread of brucellosis As there is no human vaccine, veterinarians and other high occupational risk persons should be made aware to take all the protective measures while handling abortions, still births, suspected infected materials and samples etc. In research and diagnostic laboratories, *Brucella* spp. should be handled under conditions of biosafety level 3 or higher. The impact of brucellosis in control programs and the consequence of their subsequent neglect has been demonstrated in Iran where incidence of animal brucellosis declined from 44% (1956) to 5% (1958)after taking suitable control program (Refai *et al.*, 2002).

In endemic areas good hygiene measures including protective clothing/equipment are very important in preventing and reducing occupational exposure. Strict precautions should be taken to avoid contamination of the skin, inhalation or accidental ingestion of organisms when assisting at a birth, performing postmortem or while handling an aborted fetus or foetal membranes and fluids. Risky agricultural practices such as crushing the umbilical cord of newborn livestock with teeth or skinning aborted fetuses should be avoided. Animal vaccines of Strain 19 and *B. melitensis* Rev-1 should be handled with caution to avoid accidental injection or exposure. Adverse events have also been reported with *B. abortus* RB 51 vaccine, although it is safer than Strain 19. Occasionally, persistent infections after vaccination have been reported in domestic animals and animals may shed strains in the milk or aborted fetuses and can infect humans.

Once brucellosis is suspected or diagnosed in a herd or farm, identification of infected animals, eradication of the infected and carrier animals, preventive steps to inhibit transmission to healthy and susceptible as well as measures to prevent re-introduction of the diseased animals need to be done.

The disinfection of premises and equipments plays an important role as a preventive and control measure. *Brucella* species are readily killed by most commonly available disinfectants including 70% ethanol, phenolic disinfectants, formaldehydes, hypochlorite solutions, isopropanol, iodophores, and xylene; however, their efficacy decreases in presence of organic matter and low temperatures. *Brucella* contaminated surfaces can be disinfected by 2% formaldehyde solution, 2.5% sodium hypochlorite, 20% freshly slaked lime suspension and 2-3% caustic soda. Autoclaving clears contaminated equipment from *Brucella.* Gamma irradiation (e.g. in colostrum) and pasteurization also destroys *Brucella*. It is reported to persist for weeks in ice cream and for months in butter. This organism survives for very short periods in meat unless it is frozen.

Diagnostic aspects

Diagnosis of brucellosis is a challenging task and often delayed or missed because clinical picture may mimic other infectious or non-infectious diseases (Araj, 1999) especially in young and non-pregnant female bovines, bulls and humans. Isolation and identification of *Brucella* from clinical and morbid materials is possibly the most reliable method for diagnosis in domestic animals (Gwida *et al.*, 2010).Diagnosis is mostly done by milk ring test, serological tests and bacterial isolation. Although several polymerase chain reaction (PCR) assays have been developed, serological tests (serum agglutination test, SAT; Rose Bengal test, RBPT; complement fixation test, CFT; and enzyme-linked immunosorbent assay, ELISA) are still frequently used as diagnostic methods (Gwida *et al.*, 2010). All tests have limitations concerning specificity and sensitivity especially when testing individual animals.

Although a high percentage of animals exhibited sero-positivity, bacterial isolation was low due to slow growth of *Brucella* spp. and the growth of these organisms depend upon stage of the disease, type of culture medium, putrefaction of specimen, overgrowth of contaminants, quantity of bacteria and culture technique employed. The "gold standard" in the diagnosis of brucellosis is bacterial isolation, which requires long cultivation periods and is often unsuccessful. Isolation and identification fail in a surprisingly high proportion of cases.

Samples to be taken for diagnosis

Milk and serum samples from pregnant and non-pregnant animals and serum from male animals are used for serological testing. Many samples can be taken from aborted foetuses (placenta, stomach contents, abomasal content, fetal heart, spleen, lung and kidney), fetal fluids and membranes, as well as vaginal discharges for isolation of *Brucella* organism. Other secretions and excretions including semen, urine and hygroma fluids can also contain organisms. Bacteria are also found in the bursa of horses infected with poll evil or fistulous withers. At necropsy, bacteria can be isolated from a variety of organs including lymph nodes, spleen, uterus, udder, testis, epididymis, joint exudates, abscesses and other affected tissues.

Vaccination and its limitations

Calfhood vaccination is generally done at the age of 4-6 months in female animals with *Brucella* S-19 vaccine. *Brucella* strain 19 vaccine has already proven a way for controlling the disease in developed countries. The antibodies after vaccination persist up to 6 months and later it disappears by 8 months. Thereafter, the animal may remain antibody free provided not revaccinated.

However, as brucellosis transmitted from small ruminants poses a significant health risk factor, efforts are urgently required to control brucellosis in sheep and goats where *B. melitensis* infection is problematic and *B. abortus* vaccine do not protect effectively against *B. melitensis*. *Brucellamelitensis*Rev1 vaccine has not yet evaluated for use in cattle. In small ruminants the initial step in brucellosis control is to vaccinate young animals (kept as replacements) with the *B. melitensis* Rev.1 vaccine. This approach is based on the hypothesis that the Rev1 vaccine offers life-long immunity and that after implementing the vaccination program for 5-7 years, which is the reproductive life-span of sheep and goats, the whole population will be vaccinated and fully protected against brucellosis. This method is also recommended to minimize hindrances in diagnosis and to prevent abortion after vaccination. In many countries, the use of *B. abortus* strain vaccine in cattle and *B. melitensi s*strain Rev1vaccine in sheep and goats has resulted in the elimination or near-elimination of brucellosis in these animals.

A plan for the control of bovine brucellosis has already been developed in India (Renukaradhya *et al.,*2002). Also, the Government of India has made it mandatory to regularly screen all the breeding bulls from artificial insemination centers for brucellosis and to use brucellosis free bulls for semen production. 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. A paradigm shift in our approach from the current 'biomedical model' to a 'sociocultural model' is needed for the control and elimination of brucellosis in India.

In spite of the clinical efficacy and cost effectiveness of vaccination of livestock, the limited availability of vaccines and lack of awareness have led to the persistence of brucellosis in rural areas of India. Till date, no vaccine is available for the prevention of brucellosis in humans. As genomic sequences of *B. melitensis*, *B. abortus* and *B. suis* have been determined and this improved understanding of the biology and pathogenicity will be of immense use in developing new acellular and safer vaccines to control brucellosis (Del Vecchio, 2002; Sanchez *et al.,* 2001).

Treatment aspects

Brucella spp are facultative and intracellular pathogens and they are inaccessible to antibiotics and clinical efficacy of antibiotics does not always correlate with *in vitro* susceptibility (Hall, 1990). For better management of brucellosis in livestock and owing to economic reasons, the treatment is not recommended unless a particular animal is highly valued. In view of antibiotic resistance of isolates of brucellae in nature, their public health concerns and occupational risk, a carefully thought-out decision must be made before recommending any antibiotic therapy. Only scant references are available with regard to treatment of brucellosis, both in humans and animals (Verma *et al.*, 2000). Different antibiotic susceptibility is reported by some authors. The pharmacokinetics of chemotherapeutics against brucellosis in different species of livestock does not appear to have been undertaken. The biological half-life of such chemotherapeutics in uterus needs to be assayed both in clinical and carrier animals.

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