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Original Article

Ovine and Caprine Brucellosis in India

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Abstract

Brucellosis is one of the major bacterial disease that affects almost all livestock species and it poses an important zoonotic threat in humans of all ages. In India, ovines and caprines are an important source of livelihood for majority of the agricultural people. Increased demand for meat, dairy and dairy products resulted in excessive farming practices and lead to the spread and transmission of the disease.

In sheep and goat the disease is characterised with abortions and infertility. Serological and molecular tests were employed for the diagnosis of disease in ovineand caprine. Prevention is done by mass vaccination of suspected and infected animals. But, In humans the disease is difficult to diagnose and it is time taking process and requires laboratory testing for confirmation and it necessarily requires awareness within humans to take preventive measures in controlling the spread of disease.

Keywords: Brucellosis; Ovine; Caprine; Abortions; Zoonotic; Serological and Molecular Diagnosis.

Introduction

Brucellosis is an endemic disease in India [4] caused by a Gram negative bacterium and has world wide importance as it affects a variety of animal species and humans too. Brucella are obligatory intracellular parasites which require an animal host for its survival. The organisms localize in the reticuloendothelial system and genital system. Usually in animals,thedisease is characterized commonly by abortions in females and orchitis in males associated with chronic infections leading to debilitating conditions. The disease was first reportedin 1887 by David Bruce, who isolated *Brucellamelitensis* (Micrococcus melitensis at that time) from the spleen of a British soldier who died from a febrile illness (Malta fever) common among military personnel stationed in Malta. Later after 20 years, in 1905 Zammit observed the zoonotic nature of *B. melitensis* from goat's milk. It was found that healthy goats acts as carriers of the disease [30,37]. In 1897, a Danish veterinarian, L.F. Benhard Bang, discovered Bang's bacillus or bacillus of cattle abortion (B.abortus) to be the causative agent of Bang's disease. Alice Evans, worked on pathogenic bacteria isolated from dairy products and they found a relationship between Bang's disease and Malta fever and in honour of David Bruce they named the genus Brucella.

The disease has been endemic in India with a seroprevalance rate ranging from 6.5% to 16.4% different livestock species. Many factors like failure to vaccinate young female calves, no proper implementation of test and slaughter procedure, absence of a proper treatment and practice of selling an affected animal to other farmers all play a vital role in the spread and transmission of disease. In

India, the disease is considered as a serious important economic problem due to the losses in livestock and human population. The losses due to brucellosis were found to be as much as 18.7 times higher than that reported for cystic echinococcosis in livestock species in previous studies [28]. Other than in India, brucellosis is also a serious economic concern in several other countries suggesting that brucellosis causes huge economic losses in the developing countries.

The Etiological Agents

Ovine and Caprine brucellosis is caused by *B.ovis* and *B.melitensis* respectively but *B.melitensis* is more virulent and responsible for the disease in both species [16] sometimes goats can be infected with *B.abortus*also.These are facultative, intracellular gram-negative cocco bacilli, non spore and non capsulated pathogens having the ability to cause persistent infections in animals. They are placed under alpha-2 subdivision of the Proteobacteria, along with Ochrobactrum, Rhizobium, Rhodobacter, Agrobacterium, Bartonella, and Rickettsia [38]. The names of the species is given based on the animal preferentially infected [36] *B.ovis*, *B.melitensis* along with *B.abortus* are called as classical brucella as these are the most common strains in livestock causing brucellosis. *B.melitensis* is differentiated into three biovars(biovar 1,2 and 3) where as *B.ovis* is undifferentiated [8]. The genetic makeup is similar for all the members of Brucella with an average genomic size of 3.29Mb and two cicular chromosomes. Chromosome I is nearly 2.11 Mb and chromosome II is1.18 with G+C content of 57.2% and 57.3% respectively [7,17,24].

World wide Distribution

First identified in Egypt later its occurrence has been throughout the world except in countries where bovine brucellosis (B.*abortus*) has been eradicated. The countries include Australia, Canada, Cyprus, Denmark, Finland, The Netherlands, New Zealand, Norway, Sweden and the United Kingdom. The *B.melitensis* distribution is associated with Mediterranean littoral especially countries of Europe, northern and eastern Africa, Near east countries, India [6,13,14], Central Asia, Mexico and Central and South America are still not brucellosis free [33].

Transmission and Clinical Signs

Brucella organism may either enter the body through the digestive tract,lungs or mucosal layers and spread through the blood and the lymphatic system to any other organ where it infects the tissue and gets localized. *Brucella* hasthe ability to replicate and persist in host cells thus causing persistent disease and to circumvent innate and adaptive immunity [12,15]. The lipopolysaccharide (LPS) of *Brucella*an external membrane component, possessing low endotoxicity, modulates the host immune response confering resistance to antimicrobial activity and acts as virulence factor for survival and intracellular replication [21]. Brucellosis is almost invariably transmitted to man from infected domestic animals affected with*B. melitensis.* The common routes of transmission mainly include placenta, aborted fetal fluids, vaginal discharges, semen and milk. About 80% of the infected animals continue to secrete the pathogen in milk throughout their lives [18]. One of the most prominent clinical sign of ovinesand caprines affected with Brucella, is abortion of dead or weak foetus usually in the second trimester [1]. Other signs include stillbirths, decreased fertility and low milk production. There is an estimated reduction upto 25% in milk and the organisms localize in the supra-mammary lymph nodes and mammary glands.

Public Health and Economic Importance

Public health and economic impact of brucellosis always remain a serious concern in developing countries due to its zoonotic importance and constraints in improving animal husbandry. *B.melitensis* is the most pathogenic species commonly involved in causing brucellosis infection with major veterinary and public health significance worldwide nearly affecting 50,000 human cases reported annually [27]. Food products of sheep and goat are one of the sources for human infections. Transmission may occur when humans come in contact with infected animals or by consumption of milk and milk products like cheese and rennet prepared from unpastuerised milk of sheep and goat [28].Human brucellosis is known for its complications and involvementof internal organs and development of symptoms depends on the site of infection. Symptoms mainly include undulant fever, abortions, sexual impotence, nervousness, depression,arthralgia,encephalitis, meningitis, spondylitis, arthritis, endocarditis, orchitis, and prostatitis [1] etc. In pregnant women spontaneous abortions in first and second trimesters are reported.A rare complication Brucella endocarditis (<2% of cases) is most commonly associated with *B. melitensis* infection results for at least 80% of deaths [25,32]. Direct spread from person to person is very

rare, but cases have been reported of the transmission through breast feeding and sexual coitus. The estimated economic losses obtained from epidemiological surveys in India revealed that brucellosis infection on livestock in India is responsible for a median loss of US $ 58.8 million per year [20]. The disease is responsible for US $ 0.7 per sheep and US $ 0.5 per goat indicating significant economic losses in India.

Diagnosis

Laboratory diagnosis plays a vital role in confirming Brucellosis. Several serological and molecular techniques have been developed for diagnosing infections in animals and humans. Accurate and fast diagnosis of human brucellosis is very necessary as delay or misdiagnosis results in failure treatments, chronic infections and high case fatality rate.

Gold standard method includes isolation of bacteria from blood, bone marrow, cerebrospinal fluids and lymph nodes on selective agars but the major disadvantage is they cannot be used as a screening test. Slow growth, poor sensitivity and many factors like quantity of pathogen,indidvdual laboratory practices act as restraints for the culturing of brucella on agars. For better and accurate confirmation of brucellosis serological methods like Rose Bengal test(RBT), counterimmuno-electrophoresis (CIEP), Coombs test,milk ring test,immunocapture agglutination test (Brucellacapt), latex agglutination, the indirect enzyme-linked immunosorbentassay (ELISA) [1]. Molecular diagnosis using polymerase chain reaction (PCR) based assays has been proposed as an useful and more sensitive tool in brucella infections.

Rose Bengal plate test , a rapid screening test for brucellosis diagnosis [34] has high sensitivity but of low specificity as sometimes it gives a false positive result [29]. To overcome these super agglutination tests were performed to get accurate tests with a sensitivity of 95.88%. But when compared to iELISA and CFT, the specificity of superagglutination was low [31].

Fluorescent polarization assay and cELISA proved to give an accurate result in the diagnosis of Brucellosis infections in livestock [23].

Lateral flow assays(LFA) for the detection of Brucellosis based on the results were found to be 100% and 90% for caprine and ovine respectively. Herds free of brucellosis tested with LFA reacted negative indicating higher specificity butnon specific results arised with positive ser samples [35].

Of all the serological tests, ELISA is considered as most sensitive and evaluation of different enyme linked immunosorbant assays for detection of *B.melitensis* revealed that positive results were obtained with competitive ELISA and blocing ELISA rather than tests conducted with indirect ELISA. It was also found that complement fixation test had higher positive rates when compared with blocking ELISA and rose bengal plate test it is best suggested for diagnosing *Brucellamelitensis* infections [22].

Studies were performed on diagnostic efficiencies and logistic analysis of serological assays and indicated that rELISA(recombinant Omp31 based ELISA and dot-ELISA is best for detection of anti *B. melitensis* antibodies in goats.Moreover for gene based detection 16s rRNA and 16s-23 rRNA gene is the besttarget for *B. Melitensis* and for species based identification Omp31 and BP26 gene targets are suitable [16].

Control Strategies

Importanceof brucellosis infection in livestock and humans is a threat for developing countries like India. It seriously impairs the socio economic development and acts as a barrier in trading of animals and animal products. There is a need to focus on the control strategies and a collective planning progamme is required for the elimination of ovine and caprine brucellosis.

The intracellular localization of the organism and its ability to survive results in failure of treatment.The combination of antibiotics doxycycline with streptomycin is the best therapeutic option for brucellosis [3,9,11,28]. In endemic regions, the only means to control and eradicate the disease is mass vaccination of susceptible hosts and removal of infected animals [5]. It is not 100% that vaccination alone can prevent the disease but in addition good animal husbandry practices are also required to succed against the disease.

B. melitensis strain Rev1 is presently the best vaccination available for the control of ovine and caprine brucellosis, Rev 1 strain shows a moderate degree of virulence and readily induces abortions when given during pregnancy and it is highly infectious to man. Further, several attempts have been made to produce B. melitensis rough live attenuated strain devoid of the O-side chain.Trail experiments in the field have to be conducted for its evaluation. [2]. At present no vaccine is available for prevention of brucellosis in humans. The only way to minimize the disease in humans is through mass vaccination of livestock together along with measures like

movement control, testing and isolation of infected animals. All these activities can collectively control brucellosis in livestock and finally aiding in reduced transmission of disease from animals to humans [19].

Brucellosis eradication programmes are very cost effective but it was estimated that for every $1 spent on eradication it saves $7 of economic losses [1,30].

Conclusion

Regular surveillance programme, well organised educational programmes to farmers about the spread of disease to animals and humans helps in a definitive control of the disease. strict and effective policy programmes are needed in educating farmers and emphasing more on the economic losses caused by brucellosis infection.

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