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International Journal of Current Research
Vol. 5, Issue, 07, pp.1920-1925, July, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

ISOLATION AND MOLECULAR DETECTION OF *Brucella melitensis* FROM DISEASE OUTBREAK IN SHEEP AND *B. abortus* FROM CATTLE FARM BY *IS711* and *omp2a* GENE BASED PCR

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ARTICLE INFO

Article History:
Received 12th April, 2013
Received in revised form
19th May, 2013
Accepted 18th June, 2013
Published online 18th July, 2013

Key words:

Brucella melitensis,
Brucella abortus,
Sheep,
Cattle,
Isolation,
Identification,
PCR.

ABSTRACT

Brucellosis is one of the world's major zoonoses that still is of veterinarian, public health and economic concern in many parts of the world. Brucellosis due to *Brucella melitensis* is of much public health and economic importance in many developing countries including India and is considered to be the major cause of abortion in small ruminants. The present study was carried out with the objective of cultural isolation of *B. melitensis* from clinical samples (knee joint fluid and aborted foetal material from 56 animals) collected from a disease outbreak in sheep in Saharanpur District (U.P., India) and its confirmatory detection using molecular tool of polymerase chain reaction (PCR). Out of a total number of 56 clinical samples, 42 (75%) bacterial isolates of *Brucella* spp. were recovered. On the basis of colony morphology, staining characters, phenotypic and biochemical characterizations, the organisms from clinically infected sheep identified as *B. melitensis*. Further confirmation of *B. melitensis* done by PCR amplification of *IS711* and *omp2a* target genes gave specific amplicons of 731 bp and 1104 bp fragment sizes, respectively for all the 42 cultural isolates obtained. In addition, using both cultural methods and PCR, 02 (33.33%) isolates of *B. abortus* were also isolated and identified from liver samples of aborted bovine foetus (n=6) collected from an organized Cattle farm of Bareilly (U.P.). Isolation and confirmatory diagnosis of *B. melitensis* and *B. abortus* indicates appropriate prevention and control strategies for this economically important pathogen having zoonotic significance.

INTRODUCTION

Brucellosis, caused by members of the genus *Brucella*, is an important re-emerging bacterial zoonosis and a significant cause of reproductive losses in animals (Corbel, 1997; Cutler and Whatmore, 2003; CDC, 2007; Gul and Khan, 2007; Radostitis *et al*., 2007). It is a major cause of disease in livestock world-wide, with substantial implications for animal welfare and economic output. The economic losses by brucellosis in animals are due to abortions, premature births, decreased milk production and repeat breeding, and may lead to temporary or permanent infertility in infected livestock, and is a major impediment for trade and export (Erdenlig and Sen, 2000; Al-Talafhah *et al.*, 2003). The disease is usually caused by *Brucella abortus* in cattle, *B. melitensis* or *B. ovis* in small ruminants, *B. suis* in pigs and *B. canis* in dogs (Corbel and Brinley-Morgan, 1984; Anon, 2001; Osterman and Moriyon, 2006). Among the different species of the *Brucella* genus, *B. abortus* and *B. melitensis* are the most pathogenic and virulent, not only for cattle, sheep and goats, respectively, but also for other animal species. It is present in all livestock systems and increased demand for dairy products accompanied with changing and intensified farming practices has raised the concern for increased spread and intensified transmission of this infection to the human population with increased risk of disease.
B. abortus is predominantly associated with bovine brucellosis. Brucellosis in sheep and goats is primarily caused by *B. melitensis*, and rarely by *B. abortus* or *B. ovis*. *B. melitensis* primarily affect the reproductive tract of sheep and goats, and it is characterized by abortion, retained fetal membrane and to lesser extent, impaired fertility. Ovine brucellosis occurs naturally only in sheep and it can infect all breeds of sheep, and is responsible for undulating or Malta fever in humans. Sporadic cases caused by *B. abortus* have been observed, but cases of natural infection are rare in sheep and goats. Cattle can also become transiently infected by *B. suis* and more commonly by *B. melitensis* when they share pasture or facilities with infected pigs, goats and sheep.

Although a few parts of the developed world have eradicated the disease by the combination of strict veterinary hygiene measures, monitoring programs and improved food safety measures, it remains endemic in large areas. Because of its high incidence in developing countries, economic consequences, and difficult eradication, the World Health Organization considers brucellosis as one of the seven neglected zoonoses, a group of diseases that contribute to the perpetuation of poverty (WHO, 2006). Brucellosis is still an uncontrolled serious public health problem in many developing countries including India (Acha and Szyfres, 2003; Saleem *et al*., 2004; Benkirane, 2006; Minas, 2006; WHO, 2006). *B. melitensis*, *B. suis* and *B. abortus* are pathogenic for man and are listed as potential bio-weapons by the Centers for Disease Control and Prevention in the USA. *B. canis* can also infect human. Of main concern in India are *B. melitensis* and *B. abortus* (Henk *et al*., 2005). *B. melitensis* is most virulent for man. The traditional methods identify species of infecting *Brucella* by isolation of bacteria on selective media followed by quantitative analysis of phenotypic and cultural/biochemical properties of the organism. However these are time consuming, laborious, and costly, and thus are not suitable for as routine diagnostics. Conventional diagnostics including serological tests also lack sensitivity and specificity. Important serological diagnostic tools

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are serum tube agglutination test (STAT), complement fixation test (CFT), Rose Bengal Plate test (RBPT) and enzyme linked immunosorbant assay (ELISA). Recently various molecular techniques have been developed for the rapid detection of *Brucella* in animals and human beings. These techniques are more sensitive and specific, quick to complete, and often can be automated to accommodate minimal labour and/or high throughput (Ignacio and Ignacio, 2004). Despite the high degree of DNA homology within the genus *Brucella*, several molecular methods, including PCR (Mirnejad *et al*., 2012), PCR restriction fragment length polymorphism (PCR-RFLP) (*omp2a*) and Southern blot, recent developments like LAMP (Song *et al*., 2012) have allowed differentiation between *Brucella* species and their biovars (Al Dahouk *et al*., 2003; Cloeckaert and Vizcaino, 2004; Sakran *et al*., 2006; Huber *et al*., 2009; Yu and Nielsen, 2010). In India, brucellosis is endemic throughout the country, and epidemiological evidence shows that brucellosis is present in different species of mammalian farm animals including cattle, goats, buffalo, yaks, camel, horses and pigs (Renukaradhya *et al*., 2002; Radostits *et al*., 2007). *B. abortus* biotype 1 in cattle and buffaloes and *B. melitensis* biotype 1 in sheep, goats, and humans are the predominant infective biotypes in the country (Radostits *et al*., 2007). The present study aimed at cultural isolation and molecular detection of *Brucella* spp. from clinical samples collected during field investigation from a disease outbreak in sheep and from aborted bovine foetus from an organized Cattle farm by employing *IS711* and *omp2a* gene based PCR assay, which is a rapid and confirmatory diagnostic tool.

MATERIALS AND METHODS

Bacterial Strains

Field isolates of *Brucella* species obtained during disease outbreak investigations of ovine brucellosis in sheep, along with isolates obtained from cases of abortions in cattle, reference strain of *B. melitensis* 16M (provided by National *Brucella* Laboratory, IVRI) and vaccine strain of *B. abortus* S19 (provided Division of Biological Product, IVRI) were used in the present study.

Sample Collection

During a disease investigation study of a suspected outbreak of ovine brucellosis in sheep at Nukad Tehsil, Saharanpur District of Uttar Pradesh, clinical materials were collected from affected animals (n=56) of different herds in the area. The main clinical manifestations of brucellosis in sheep were reproductive failures, heavy number of abortions and birth of weak offspring. Abortion generally occurred during the last 2 months of pregnancy followed by obvious signs of mastitis. Knee joint fluid samples from sheep with clinical signs of joint swelling and abortion were collected aseptically for the study. The aborted foetal materials (abdominal fluid, liver, and spleen) were collected in sterile container and in Amies transport medium, stored in icepack and immediately transferred to the laboratory. In addition, liver samples of aborted bovine foetus were collected from an organized Cattle farm of Bareilly (U.P.) region reported to be having abortions.

Bacteriological Examination

All the representative 56 clinical samples (knee joint fluid, foetal materials - abdominal fluid, liver, and spleen) collected from the affected sheep and 06 samples of aborted bovine foetus as above indicated were processed for cultural isolation of *Brucella* spp. The fluid samples were immediately inoculated aseptically to blood agar and *Brucella* agar without *Brucella* selective supplement. Isolation and identification of *Brucella melitensis* was done as detailed in Bergey's Manual of Systemic Bacteriology (Corbel and Brinley-Morgan, 1984) and OIE (2000). The aborted foetal contents (liver and spleen) were crushed into pieces with sterile mortar and pestle in PBS (pH 7.2) and streaked on to blood agar and *Brucella* agar without *Brucella* selective supplement. The agar plates were incubated at 37°C in an atmosphere of 5-10% CO_2 for 72 hrs. A replica plate was also kept at 37° C without CO_2 tension. Further identification of the organism was done by their morphology and microscopic examination using Hucker's modified Gram stain method (1923) and modified Ziehl-Neelsen stain technique outlined by Stamp *et al* (1950). Biochemical characteristics *viz* Oxidase test (Carter and Cole, 1990), Catalase test, urea hydrolysis, nitrate reduction test and hydrogen sulphide (H_2S) production, growth in the presence of thionin and basic fuchsin dyes at three different concentrations (10-40 µg/ml) (Corbel and Brinley-Morgan, 1984) were carried out.

PCR detection of *Brucella melitensis*

Genomic DNA Extraction

Extraction of genomic DNA was performed from bacterial cultural isolates of joint fluid and aborted foetal samples following CTAB method described by Wilson (1990) with slight modifications. Briefly, about 2 to 3 loops of growth from pure *Brucella* culture freshly grown on selective *Brucella* medium were transferred to a microfuge tube containing 400 µl of 1x TE buffer. The cells were killed at 80ºC for 20 min in a water bath followed by cooling at room temperature. Bacterial cell membranes were then disrupted by adding 70 µl of 10% SDS solution and 5 µl of 10 mg/ml proteinase-K and followed by incubation at 65ºC for 10 min after brief vortexing. Following incubation, 100 µl of each of 5M NaCl and pre-warmed CTAB-NaCl solution was added. The mixture was vortexed until the liquid became milky white and incubated at 65ºC for 10 min. Subsequently, 750 µl of phenol: chloroform: isoamyl alcohol (25:24:1) was added, vortexed briefly and then centrifuged for 8 min at 11,000 x g. The aqueous phase containing DNA was carefully transferred to a fresh microfuge tube and the DNA was precipitated by addition 0.6 volume of isopropanol. The tubes were then kept in –20ºC for 30 min followed by centrifugation for 15 min at 11,000 x g. The supernatant was discarded, leaving about 20µl above the pellet, which was then washed with 1 ml of cold 70% ethanol and centrifuged for 5 min at 11,000 x g. After discarding the supernatant, the pellet was subjected to drying at room temperature for 15-30 min and finally dissolved in 20 to 30µl of 1x TE buffer and was stored at –20ºC until further use.

PCR Amplification

Genomic DNAs extracted were used to amplify target *Brucella* specific genes (*IS711* and *omp2a*) by PCR. Oligonucleotide primers specific for *IS711* and *omp2a* gene of *B. melitensis* and *B. abortus* were used to amplify a fragment size of 731 bp and 498 bp for IS711 (Khosravi *et al*., 2006), and 1104 bp (Vivekananda *et al*., 2009) and 966 bp (Paquet *et al*., 2001) for *omp2a*, respectively (Table 1). PCR assay was performed in a final volume of 25 µl mixture containing 10x PCR buffer with 1.5 mM $MgCl_2$ (2.5 µl), 0.2 mM of each deoxynucleotide triphosphate (0.5 µl), 1.25 unit Taq DNA polymerase (0.4 µl) (Banglore Genei, India), 0.5 µmol of each primer (0.5 µl, MWG Biotech, Germany), and 3 µl template DNA. Positive control using template DNA from reference strains and negative control, containing all the reagents but nuclease free water in place of template DNA was included in all experiments. The amplifications were carried out with 30 cycles, and PCR conditions were standardized as presented in Table 2. The PCR products were analyzed using 1.2% (w/v) agarose gel with 0.5 µg/ml ethidium bromide (Banglore Genei, India) using 1x TAE electrophoresis buffer.

RESULTS AND DISCUSSION

Brucellosis has been an emerging disease since the discovery of *Brucella melitensis* by Sir David Bruce in 1887. Ovine brucellosis has been reported in most of the major sheep producing regions of the world and is present in Australia, New Zealand, the United States, South America, central Asia, South Africa and Europe. Brucellosis is perhaps the most widespread and economically important of the zoonotic diseases in tropical and subtropical regions (Gul and Khan,

Table 1. Details of primers used in the study

Brucella sp.	Gene (Size in bp)	Primer	Sequence
B. melitensis	*IS711* (731)	BMF	5'- GAC GAA CGG AAT TTT TCC AAT CCC-3'
		BMR	5'-TGC CGA TCA CTT AAG GGC CTT CAT-3'
B. abortus	*IS711* (498)	BAF	5'-AAA TCG CGT CCT TGC TGG TCT GA-3'
		BAR	5'-TGC CGA TCA CTT AAG GGC CTT CAT-3'
B. melitensis	*omp2a* (1104)	*omp2a* F	5'-TCT CCT TGG CTC CGC TGC A-3'
		omp2a R	5'- CGA ACG ATA CCG CCC CAG G-3'
B. abortus		*omp2a* F	5'-ACG GCG CTG GCT ACT TCT A-3'
	omp2a (966)	*omp2a* R	5'- TTA GAA CGA GCG CTG GAA G-3'

F= Forward, R= Reverse

Table 2. Standardized PCR conditions for *IS711* and *omp2a* genes based detection of *Brucella*

Specie	Gene (size)	PCR conditions			
		Denaturation	Annealing	Extension	Final extension
B. melitensis	*IS711* (731)	94 °C for 1 min	58 °C for 1min	72 °C for 1 min	72 °C for 10 min
	omp2a (1104)	94 °C for 1min	57 °C for 1min	72 °C for 1 min	72 °C for 5 min
B. abortus	*IS711* (498)	94 °C for 45sec	59 °C for 45sec	72 °C for 30 sec	72 °C for 5 min
	omp2a (966)	94 °C for 1min	63 °C for 1min	72 °C for 1min	72 °C for 7 min

2007). Raw milk and cheese products from infected goats and sheep provide a risk for human and were the mechanism for the occurrence of Malta fever that initiated the definition of the disease (Radostits *et al*., 2000). In the present disease investigation of a suspected brucellosis outbreak in sheep, both cultural isolation and identification procedures as well molecular detection by PCR of the causative agent were employed. Out of a total number of 56 clinical samples of sheep and 06 clinical samples of cattle subjected to cultural isolation, 42 (75%) and 02 (33.33%) bacterial isolates of *Brucella* spp. were recovered, respectively. Field isolates of *Brucella* species were maintained in serum dextrose agar and *Brucella* selective medium to isolate and identify *B. melitensis.* A characteristic of *Brucella* growth with very small, glistening, smooth, round and pin-point like colonies with honey like appearance were observed on Blood and *Brucella* selective agar plates after 72 hrs incubation at 37°C. Similar observations were also recorded by Corbel and Morgan (1984). The organisms did not grow on MacConkey agar, Muller Hinton agar and found to be non-hemolytic on blood agar. These exclude rapidly growing, hemolytic or lactose fermenting organisms. Microscopic examination of Gram stained cultures revealed small Gram negative coccobacilli arranged singly, in pairs or groups and on Modified Ziehl-Neelsen (MZN) stain, the *Brucella* organisms were stained red against a blue background. On different biochemical reactions, *Brucella* organisms were found to be positive for catalase, oxidase, urea hydrolysis and nitrate reduction tests and negative for indole production, citrate utilization, Methyl red and Voges-Proskauer tests.

Similar findings were reported in different isolates of *Brucella* species by Koneman *et al*. (1997) and Erdenlig and Sen (2000). On the basis of colony morphology, staining characters, and biochemical tests, the organisms were identified as *Brucella* species. The isolates were further differentiated phenotypically into species and partially to biovars using parameters such as CO_2 requirement, H_2S production, and growth on media plates containing thionin and basic fuchsin dyes at three different concentrations. Accordingly, *Brucella* species growing on tryptic soya agar media containing both thionin and basic fuchsin dyes at concentration of 10 µg/ml, 20 µg/ml, and 40 µg/ml were considered as *Brucella melitensis* where as isolates with no growth at all concentrations in both the cases (thionin and basic fuchsin) were considered as *Brucella abortus* biovar 2 and those growing on media with thionin at only 40 µg/ml (1:25,000) concentration and basic fuchsin at all concentrations were considered as *Brucella abortus* biovar 3. Requirement of CO_2 has been observed in the two biovars of *Brucella abortus,* but not in *B. melitensis*. This was in agreement with the reports by Morgan (1961), Corbel (1991), Anon (2001), and Stack and MacMillan (2003). Absence of growth on media containing streptomycin (2.5µg/ml) and requirement of CO_2 by field isolates of *B. abortus* differentiated the organisms from vaccine strains *B. melitensis* Rev1 and *B. abortus* S19, respectively. The different species and biovars of *Brucella* have been characterized according to growth behaviour on different media, CO_2 requirement, H_2S production, growth in the presence of dyes (thionine and basic fuchsin), reaction with monospecific A and M antisera, bacteriophage typing (Alton *et al*., 1988; Corbel, 1991; Muz *et al.*, 1999). All the cultural isolates obtained in the present study from clinical samples of sheep and cattle, were identified to be *B. meltinensis* and *B. abortus*, respectively. Brucella being a small, gram-negative coccobacillus that grows slowly *in vitro*, therefore these traditional methods require several weeks time to complete, and are laborious, costly and cannot routinely be used as a diagnostic procedure in developed or developing countries (Stemshorn, 1984; Al Dahouk *et al*., 2005; Elfaki *et al*., 2005). In addition, standard serological tests used to detect *Brucella* are also time consuming, lack sensitivity and specificity, and are not able to distinguish between species of *Brucella* (Gurturk *et al*., 2000; Ongor *et al*., 2001; Elfaki *et al*., 2005). Therefore, the field isolates obtained in the present study from the affected sheep and cattle were further subjected to rapid molecular technique of PCR detection for final identification and confirmation of *Brucella* spp.; *Brucella melitensis and B. abortus*, respectively. The genomic DNA extracted from all these field isolates of *Brucella* were used to amplify targeted and specific bacterial genes, *IS711* and *omp2a.* A PCR product of about 498 bp size was obtained from two *Brucella abortus* isolates and about 731 bp size from *Brucella melitensis* isolates using IS711 insertion sequence as a target gene (Fig. 1).

Similarly with a specific primer to an outer membrane protein 2a (*omp2a*), an amplified product size of about 966 bp was obtained from the two isolates of *B. abortus* and about 1104 bp from the *B. melitensis* isolates (Fig. 2 and 3). Thus all the respective field isolates obtained from affected sheep and cattle were identified and confirmed as *B. melitensis* and *B. abortus* isolates based on cultural characteristics, biochemical profiles, partial bacteriological biotyping techniques and the species specific PCR assay. These results were in accordance with the reports of Paquet *et al.* (2001), Khosravi *et al*. (2006), and Vivekananda *et al.* (2009) as they showed similar amplified products in different isolates of *Brucella* species. Molecular techniques like the PCR and RFLP are needed to differentiate species and strains within the genus *Brucella* (Al Dahouk *et al*., 2003). PCR is potentially a useful method on samples containing a low number of brucella and has potential to be a promising tool for the diagnosis of acute disease (Kolar, 1984; Sakran *et al*., 2006). Differentiation of different species viz., *Brucella abortus* bv 1, 2, and 4, *Brucella melitensis, Brucella ovis* and *Brucella suis* bv. 1 by PCR has been reported (Bricker and Halling, 1994). Recently, Huber *et al*. (2009) also emphasized the development of a

PCR assay for typing and subtyping of Brucella species, and supported PCR to be very useful during disease investigations and studying epidemiology of *Brucella* infections in animals and humans. IS711 provides a specific target able to generate data on DNA polymorphisms among the brucellae. PCR-based methods are more useful and practical than conventional methods used to identify *Brucella* spp. Recently, the PCR-based methods for identification of *Brucella* in biological samples has been reviewed with emphasis on using single-pair primers, multiplex primers, real-time PCRs, and PCRs for molecular biotyping, which have been suggested to be very important and rapid tools for *Brucella* identification, at the species level and also at the biovar level (Yu and Nielsen, 2010). Also, these techniques require minimum biological containment, and genetic fingerprinting of isolates aid in epidemiological studies of the disease and its control. There is no single test by which a bacterium can be identified as *Brucella*. A combination of growth characteristics, serological, bacteriological methods and/or molecular methods is usually needed (OIE, 2009).

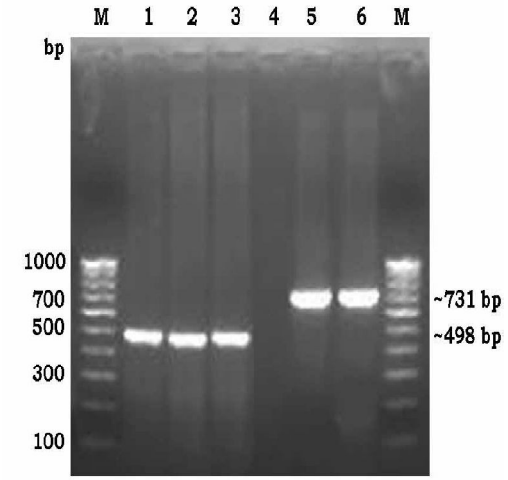

Fig. 1: PCR amplification of *Brucella IS711* gene

Lane M: 100bp DNA ladder; Lane 1: *B. abortus* isolate 1 (498 bp size); Lane 2: *B. abortus* isolate 2 (498 bp size); Lane 3: *B. abortus* S 19 (Positive control); Lane 4: Negative control; Lane 5: *B. melitensis* (731bp size); Lane 6: *B. melitensis* 16M (Positive control).

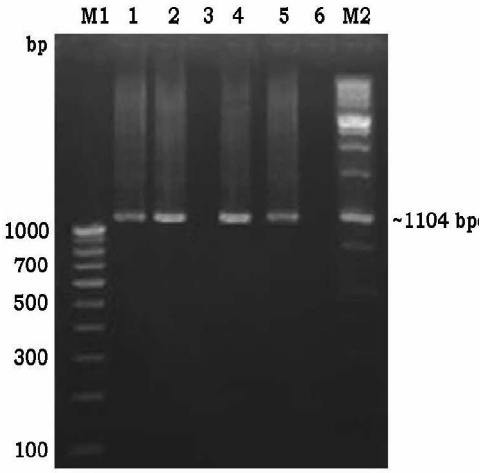

Fig. 2: PCR amplification of *B. melitensis omp2a* gene

Lane M1: 100bp DNA ladder; Lane 1 & 2: *B. melitensis* (1104 bp): Lane 3: Negative control; Lane 4 & 5: *B. melitensis* 16M (Positive control): Lane 6: Negative control; Lane M2: 1kb DNA ladder.

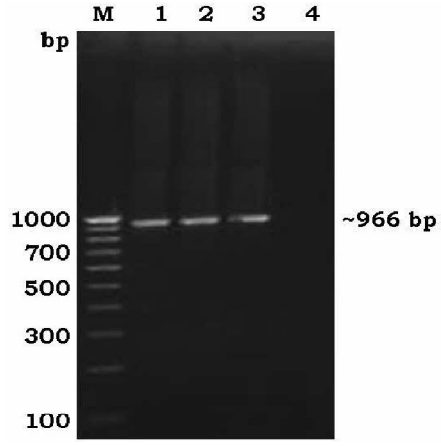

Fig.3: PCR amplification of *B. abortus omp2a* gene

Lane M: 100bp DNA ladder; Lane 1: *B. abortus* isolate 1 (966 bp): Lane 2: *B. abortus* isolate 2 (966 bp); Lane 3: *B. abortus* S19 (Positive control): Lane 4: Negative control.

Detection of *Brucella* species DNA from aborted bovine and sheep foetuses has been reported by PCR (Cetinkaya *et al*., 1999; Cortez *et al*., 2001). Utility of PCR for detection of *Brucella* species in the milk of infected cattle, sheep, goats and camels has also been reported by Hamdy and Amin, 2002. Regarding India, most of the investigations for brucellosis in bovines, sheep, goats and human infections have been based on serological surveys (Desai *et al*., 1995; Kumar *et al*., 1997; Isloor *et al*., 1998; Mehra *et al*., 2000; Singh *et al*., 2000; Sen *et al*., 2002; Henk *et al*., 2005; Sharma *et al*., 2007). Chahota *et al.* (2003) have reported a severe outbreak of brucellosis in an organized dairy farm leading to abortions, retained placenta and still birth in cows. The diagnosis was made by serology employing rose Bengal plate agglutination test (RBPT) and standard tube agglutination test (STAT) and confirmed by the isolation of *B. abortus* biotype1. Different *B. abortus* biotypes (types-1, 2, 4, 6 and 9) have been isolated from cattle (Renukaradhya *et al.*, 2002). Recent studies have indicated that human brucellosis is quiet common disease in India (Renukaradhya *et al.*, 2002; Mantur *et al.*, 2006). Economic losses are considerable in an agrarian country such as India. There is no organized and effective brucellosis control program. Plans for a large scale control program, including calfhood vaccination, are underway (Radostits *et al.*, 2007). Brucellosis in cattle seems to be associated primarily with intensive farming practices in large organised dairy farms.

In India, historically free roaming of animals and the agrarian nature of the different traditional animal husbandry systems provided ample opportunities for intermixing of livestock through grazing at common pastures and trading at local stock yards, also use of semen from unscreened bulls for artificial insemination and poor farm hygiene probably contribute to the spread and transmission of the infection. The changing and fast growing dairy industry has resulted in intensified trade and animal movements and provides a new and increased risk in spreading the infection (Henk *et al.*, 2005). Regarding zoonosis aspects of brucellosis, the major sources of infection and risk factors include occupational contact with infected animals, inhalation of airborne agents, ingestion of contaminated animal products and handling of *Brucella* isolates in laboratories are risk factors; and effective therapy requires an early diagnosis. The occurrence of the disease in humans is largely dependent on the animal reservoir and high rates of brucellosis infection in sheep and goats usually cause the greatest incidence of infection in humans (WHO, 2006). The disease may be overlooked and misdiagnosed because of the difficult diagnosis and the lack of experience with laboratory testing. In conclusion, in the present disease investigation study, *B. melitensis* was found to be the main aetiological agent responsible for causing disease outbreak (brucellosis) in small ruminants (sheep). Along with this, *B. abortus* was also identified to be causing abortion in cattle. *B. melitensis* vaccine strain Rev 1 sometimes isolated from some sheep abortions may lead to

misdiagnosis of the disease. However, the isolates in the present study were found to be different from *B. melitensis* Rev 1 strain and this finding may suggest that this vaccine strain is not the causative agent of ovine brucellosis in this region. Most surveys of brucellosis in India rely on serological test only, without isolation of *Brucella* spp. and this can be misleading for the ultimate action to be taken. Confirmatory diagnosis must be provided by the isolation and confirmatory identification of the aetiological agents, which is important to study the epidemiology of the disease. Apart from cultural isolation, confirmatory detection of *Brucella* spp. was achieved using rapid molecular tool of PCR. Being a contagious diseases, the isolation of B. *melitensis* from knee joint fluid and aborted sheep foetus materials may indicate high prevalence of *B. melitensis* infection among sheep in Saharanpur region and due to that, the disease may pose threat to animal as well as human health. Brucellosis is an important but neglected disease in India. Rapid and reliable molecular tools, sensitive and specific, easy to perform and automated detection systems for *Brucella* spp. are urgently needed to allow early diagnosis, epidemiological surveillance and adequate antibiotic therapy in time to decrease morbidity / mortality as well prevent its public health implications. Increasing demand for dairy products and protein, changing agricultural methods, and increased trade and movement of animals has caused concerns that the prevalence may increase. Therefore, there is an urgent need for the strict implementation of a control policy not only for cattle but also for small ruminants. Given the potentially huge economic and medical impact a control policy could be cost-effective. It is concluded that PCR assay might be useful in identifying animals with Brucella infection in quick time and may replace existing lengthy and laborious laboratory tests which require skill and courage to handle the zoonotic organism.

Acknowledgements

The authors are thankful to Director, Indian Veterinary Research Institute, Izatnagar for providing necessary facilities to carry out this study. This work was fully financed by the Ethiopian Government, Ministry of Education in collaboration with Indian Government, IVRI.

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