An outbreak of bovine brucellosis and its management in an organised farm

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Abstract
Brucellosis remains as an economically important bacterial disease affecting livestock and is of zoonotic significance. The present study deals with report of outbreak of brucellosis in a dairy farm and its diagnosis by serology and molecular confirmation using polymerase chain reaction. Repeated abortions and infertility problems were reported from an organized dairy farm in Thrissur district of Kerala. Serum samples from twenty-nine cows and milk samples from 9 lactating animals with history of reproductive disorders such as abortion, infertility and retention of placenta were collected and subjected to Indirect Multi- species ELISA (ID-Vet) for detecting antibodies to brucella. Vaginal discharges from two cows with history of recent abortion were collected and the DNA was extracted and subjected to Polymerase chain reaction using primers targeting IS711 gene of B. abortus. All the serum and milk samples were found to be seropositive by ELISA. An amplicon of 498 bp suggestive of B. abortus was obtained by PCR using specific primers. Following confirmation of brucellosis, vaccination was carried out in all female calves between the age of four to eight months with brucella strain 19 vaccine. Also, management advice was given to the farmer regarding segregation of seropositive animals, thorough disinfection of premise, disposal of aborted materials and pasteurization of milk before human consumption.

Keywords: Brucella abortus, Cows, ELISA, PCR, Vaccination.

Introduction
Brucellosis is one among the notifiable bacterial diseases of zoonotic importance and caused by a facultative intracellular gram-negative bacteria of genus Brucella. Bovine brucellosis is caused mainly by Brucella abortus that survives and reproduces within host phagocytic cells. These organisms develop an efficient adaptation by which they escape from recognition by the immune system by manipulating host cell physiology. Even though the classic virulence factors are not present, the organisms exert unique pathogenic mechanisms for cell invasion. Brucellosis represents a significant threat to the public health with the ability to re-emerge in new species and environmental foci.

Brucellosis causes considerable economic losses in dairy industry mainly due to late abortions or stillbirth or birth of weak calves and infertility. Following the first episode of abortion, there will be subsequent normal parturitions, although a second abortion can occur. Other problems encountered are drop in milk production, an increase in somatic cell counts and impaired reproductive efficiency.

The organism targets the mammary gland and causes multifocal interstitial mastitis. Infection in bulls is often not apparent but sometimes may show systemic signs like anorexia, depression and fever. Cattle and buffaloes are affected by mainly two biotypes: B. abortus biotype-1 and biotype-3. While biotype-1 predominates in organized farms, Biotype-3 is mostly isolated from villages. The presence of LPS- O chain enables the smooth strains to efficiently invade host cells making it an important virulence factor. Recent reports revealed that brucellosis causes a total economic loss of Rs. 350 million in India.

Increased trade and movement of animals and the practice of natural service in rural areas resulted in the widespread prevalence of the disease. Introduction of infected animals and subsequent abortion leads to contamination of pasture and water sources with the excretions or aborted products. Inhalation and ingestion are the important routes of transmission.

Rapid and accurate diagnosis is fundamental for control and eradication of brucellosis. Microbial, serological and molecular techniques are the three currently employed approaches for diagnosis of brucellosis. Brucella spp are class III pathogens and culture techniques would pose a potential hazard of infection. Culture provides the definitive diagnosis of brucellosis and is considered as the gold standard method, but because of laborious procedure and its health hazard, the serological tests are the most widely used tools for diagnosis. Though serological techniques have the disadvantage of cross reactivity, they are used indirectly to prove the diagnosis. A number of serum and milk-based ELISA kits are available commercially. The diagnosis of brucellosis is challenging as culturing of brucellae and seroconversion is time consuming.

Therefore, molecular techniques like PCR are promising alternatives for the diagnosis of infectious diseases caused by fastidious or slowly growing microorganisms such as brucellae. Since the source of human infection is mainly the domestic or wild animal reservoirs, prevention of human zoonotic brucellosis depends predominantly on the control of the disease in animals.
Brucellosis is endemic in India and is reported from cattle, buffaloes, pigs, sheep, goat, yaks and dogs.\textsuperscript{21,26} There are several reports of seroprevalence of bovine brucellosis from Kerala.\textsuperscript{20,22} The present study deals with report of outbreak of brucellosis in a dairy farm, its serological diagnosis and molecular confirmation using polymerase chain reaction.

**Material and Methods**

Repeated abortions and infertility problems were reported from an organized dairy farm in Thrissur district of Kerala. Detailed information regarding number of animals aborted and the time of abortion and history of other infertility problems were collected and documented. Blood samples from twenty-nine cows with a history of reproductive disorders such as abortion, infertility and retention of placenta were collected and sera were separated. Milk samples were also collected from 9 lactating animals among these 29 cows. Vaginal discharges from two recently aborted cows were collected aseptically in sterile containers with necessary precautions.

Serum samples and milk samples from the animals were subjected to Indirect Multi- species ELISA (ID-Vet) for detecting antibodies to brucella according to the manufacturer’s protocol. The diluted serum samples and positive and negative controls were transferred using a multichannel pipette (Finnpipette F2, Thermo Scientific) to an ELISA microplate coated with purified \textit{Brucella abortus} LPS. The ELISA plate was incubated at 21 °C (± 5 °C) for 45 minutes. The wells were emptied and each well was washed 3 times with 300 μl of 1X wash solution using an ELISA plate washer (Well wash, Thermo Scientific). Hundred microliters of 1X conjugate were added to each well.

The plate was incubated at 21 °C (±5 °C) for 30 minutes. The wells were emptied and each well was washed 3 times with 300 μl of 1X wash solution using an ELISA plate washer (Well wash, Thermo Scientific). Hundred microliters of substrate solution were added to each well. The plate was then incubated in the dark at 21 °C (±5 °C) for 15 minutes. Hundred microliters of stop solution were added to each well in order to stop the reaction. Optical densities were measured at 450 nm using ELISA plate reader (Varioskan Flash, Thermo Fisher Scientific) and SkanIt Software 2.4.5 RE for Varioskan Flash.

Vaginal discharges collected from two cows with history of recent abortion was processed for DNA extraction using high pure DNeasy blood and tissue DNA extraction kit (Qiagen, Germany). The extracted DNA was stored at -20°C until further use. Polymerase chain reaction was performed using primers, forward primer 5'--TGCCGATCCTTAAAGG GCCCTTCAT-3' and reverse primer 5'--GACGAA CGGAATTTTCCA ATCCC – 3' targeting IS711 gene of \textit{B. abortus}.\textsuperscript{3} Amplification conditions were: 3 min at 95 °C, 35 cycles each of 95°C for 90 s, 65 °C for 1 min and 72 °C for 1 min with the addition of a final extension period of 5 min at 72 °C. The PCR amplicons were visualized by electrophoresis in 1.5% agarose gel stained with ethidium bromide under UV transillumination. Results were captured and recorded using a digital documentation system.

**Results and Discussion**

There were 170 animals in the farm including 85 lactating cows. History of reproductive disorders such as abortion, infertility and retention of placenta was reported in 29 cows. History of abortion was reported in 18 animals in which two animals had aborted twice. Serum samples from all 29 animals were positive for brucella antibodies by ELISA. Milk samples from nine cows also showed positive reaction in ELISA. Serological diagnosis of brucellosis is used in many countries as the criteria for control and eradication of this disease. Indirect ELISA proved to be a highly sensitive and specific test for detection of antibodies in serum and milk from affected animals and also can be adapted to process a large number of samples.\textsuperscript{24,27} Godfroid et al\textsuperscript{8} reported indirect ELISA as the most useful test to detect \textit{B. abortus} persistently infected animals. Rapid and accurate diagnosis of brucellosis is essential for a positive outcome of eradication and monitoring programmes. Serology is routinely used for diagnosis of the disease in most of the areas owing to the cost effective and widely used test.\textsuperscript{11,15,16} The most economical and most widely used laboratory tests in diagnosis of the disease are the agglutination tests\textsuperscript{12,17} but the interpretation of their results is largely subjective. Bovine brucellosis is highly prevalent in India and causes significant economic losses to the livestock industry.

Brucellosis was also confirmed by PCR using DNA extracted from the vaginal discharge from recently aborted cows. An amplicon of 498 bp was obtained by PCR using \textit{B. abortus} - specific primers (Fig.1). The cultural isolation of \textit{Brucella} spp. from blood or tissues is the most confirmatory test. In addition to bacteriological examination, molecular methods like PCR are used in many laboratories.\textsuperscript{5,7} The PCR test is more important in animals as well as humans with clinical signs of disease but with negative serological tests, allowing the rapid confirmation of the diagnosis. Brucellosis is a disease of economic importance which adversely affects the reproductive and productive potential of the affected animals resulting in loss of calves, infertility as well as reduction or complete loss of milk yield.

However, Akhtar and Mirza\textsuperscript{2} reported that the calves of affected cows might acquire the infection, although the rate of seroconversion among such calves is not significant. Even though brucellae are reported to be host specific different biotypes of \textit{B. melitensis} and \textit{B. abortus} has been reported in cattle.\textsuperscript{4} Brucellae are highly pathogenic and insidious in nature causing a range of disease syndromes in humans from symptomless carrier stage to undulant fever characterized by anorexia, nocturnal perspiration, malaise, depression,
fatigue, loss of body weight and muscle aches. There are reports of brucellosis among in-contact humans both from India and abroad. The prevention of human infection is mainly dependent upon control and elimination of animal infection.

Based on the results of study, calf hood vaccination was carried out in all female calves between four and eight months of age. Management advice was given to the farmer which comprises of segregation of seropositive animals through disinfection of premises, proper disposal of placenta and aborted materials and pasteurization of milk before human consumption. In our country, vaccination is the most practical and an effective approach for the control of brucellosis. Calf hood vaccination not only controls the disease outbreak as such but can also minimize the aftereffects such as retained fetal membranes, infertility, metritis etc. and the costs incurred in the treatment of the same.

Factors responsible for outbreak of brucellosis in most of the Indian states include ignorance of carrier animals, ineffective test and slaughter policy, improper and unplanned vaccination, absence of effective quarantine and uncontrolled trans-state migration of animals.

Deepthi et al reported higher seroprevalence of brucellosis in unorganized farms than organized farms and suggested that the irregular screening of animals and introduction of animals without screening into the herd were found to be the main culprits.

Segregation and quarantine of affected animals can limit the infection but is considered uneconomical to house diseased and non-productive animals. Careful herd management, hygiene and vaccination are the three components for control and management of animal brucellosis. However, in India, only “test and segregation” policy is practically adaptable to control the disease in conjunction with efficient preventive measures and control of animal movements. Proper and hygienic disposal of uterine discharges, foetus and foetal membranes should be ascertained. Health education of farmers for increased public awareness is also necessary for controlling this zoonotic disease.

Conclusion

The present study deals with report of outbreak of brucellosis in a dairy farm and its diagnosis by serology and molecular confirmation using polymerase chain reaction. Brucellosis was diagnosed in an organized dairy farm with history of repeated abortions and infertility problems. Molecular confirmation was done using species specific PCR for B. abortus. Careful herd management, hygiene and vaccination are adopted systematically for the control of brucellosis since it is having a zoonotic significance.

References


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