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Detection of *Brucella* Species from Serum and Aborted Materials and Differentiation of Vaccine from Field Strain by Polymerase Chain Reaction in Buffaloes

Malik Raies Ul Islam[1]*, Gursimran Filia[2], Mohinder Pratap Gupta[2]

[1]Krishi Vigyan Kendra, Srinagar, Sher-e- Kashmir University of Agricultural Sciences and Technology, Kashmir – 191111, J&K, India
[2]Animal Disease Research Centre, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana – 141001, Punjab, India

ABSTRACT

The present study was aimed to assess the suitability of polymerase Chain Reaction (PCR) for detection of *Brucella* species from serum and aborted materials along with the differentiation of vaccinated animals from the animals infected with field strains. The samples collected included nine abomasal contents of the aborted foetus and 178 sera samples. All the samples were processed and DNA was extracted for detection and differentiation of *Brucella* strains. PCR detected amplicons of 193-bp in 6 (66.66%) samples of aborted fetuses and 68 (38.20%) sera samples. In all the six positive fetal stomach contents, *Brucella abortus (B. abortus)* was detected. However, serum samples positive for *Brucella* genus PCR, failed to yield positive results in species specific PCR. DNA from six *B. abortus* positive samples when subjected to Bruce ladder PCR resulted in amplification of five fragments of 1682, 794, 587, 450 and 152 bp in size. However, *B. abortus* S-19 DNA did not produce 587 bp fragment common to *Brucella* strains tested. Keeping in view, the challenges associated with conventional assays and isolation of agent, molecular methods especially PCR is a sensitive, specific and robust alternative in achieving an accurate diagnosis and differentiation of the agent especially in animals, in which this disease has an obscure nature.
Key words: Brucellosis, Buffaloes, PCR, Vaccine, Field strains.

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INTRODUCTION

Brucellosis, endemic in most parts of the world, is caused by gram-negative bacteria of genus *Brucella* (Ducrotoy *et al.,* 2016). The disease in cattle is predominantly caused by *B. abortus* and infrequently by *B. melitensis* (OIE, 2008). The organism is transmitted through abraded skin, conjunctiva, mucosa and lungs; however, oral route is the main portal of its entry into the host (Khan and Zahoor, 2018). Infections in animals frequently result in abortions and diminished levels of milk production. Once the acute period of the disease is over, animals may exhibit little or no symptoms with subsequently localization of *Brucellae* in the supra mammary lymph nodes and such animals

*Corresponding author.
E-mail address: malikrayees@gmail.com (Malik Raies Ul Islam)
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continue to shed the pathogen in their body secretions (González-Espinoza *et al.,* 2021). Both conventional and new serological assays used for identification of brucellosis infected animals are neither 100% sensitive nor specific, which results in false test outcome (Alton *et al.,* 1988). Isolation (gold standard) of the agent is the most reliable method of diagnosing brucellosis. However, it is cumbersome, requires bio-safety level three laboratory setup, trained personnel and in addition possesses a serious threat to the lab workers (Lage *et al.,* 2008). Moreover, isolation of organism is not always feasible due to very low bacterial count in the sample, loss of viability of organisms, presence of contaminants and inhibitory factors (De Miguel *et al.,* 2011).

Molecular diagnostic tools especially polymerase chain reaction (PCR) provide an efficient, sensitive and specific method for diagnosis of brucellosis. Though, the technique is a bit complex and requires sophisticated laboratory set up, however, it has high throughput and the reaction can be completed within a day. The assay can be used alone or aided with labeled probes for the identification of *Brucellae* from culture or contaminated aborted tissues (Mahajan *et al.,* 2017). Keeping in view, the robustness and high assay performance, the present study was carried out to assess the suitability of using PCR for rapid detection and differentiation of *Brucella* organisms from serum and aborted tissue samples.

MATERIALS AND METHODS

The samples collected for the purpose of molecular diagnosis of brucellosis through PCR included nine abomasal contents of the aborted fetus and 178 sera samples. The samples were stored at -20°C till further use. Vaccine strain *B. abortus* S-19 was purchased from Indian Immunlogicals and stored at 4°C till use.

DNA Extraction

DNeasy Blood and Tissue kit from Qiagen (Germany) was used for DNA extraction from the samples. The extracted DNA samples were properly labeled and stored at -20°C till further use.

Detection of *Brucella* Species by PCR

PCR components, concentration and reactions were standardized and optimized in the laboratory. DNA amplification was performed using primers originally described by Leal-Klevezas *et al.* (1995) with modifications as described by Islam *et al.* (2013). Thermo-cycling conditions for carrying out the reaction were, initial denaturation at 94°C for 4 min, denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min. The reaction was allowed for 35 cycles and terminated with a final extension at 72°C for 3 min. For the species differentiation of positive samples the method described by (Bricker and Halling 1994) was adopted. For differentiation of field strains from vaccine strain positive samples were again subjected to another cycle of amplification. The method described by (Goni *et al.,* 2008) was followed, with one modification that the number of cycles was enhanced from 25 to 40. All PCRs were performed with the appropriate inclusion of positive and negative controls. The PCR products obtained were confirmed by allowing them to electrophoretically migrate in 1.5% agarose gel containing ethidium bromide. Samples were loaded in parallel with molecular weight markers.

RESULTS AND DISCUSSION

Although PCR requires a well established laboratory facility with special instruments, the test is highly sensitive, specific and simple to perform in relatively short time within the same day. PCR can be used in detection of this agent directly from field samples, which can be very helpful in preventing the further spread of this agent to healthy animals. Also, PCR detects DNA which is present in both living and dead *Brucella* organisms, while, culture detects only the living organisms (Wang *et al.,* 2014). A total of 187 samples (178 sera samples and 9 samples from aborted fetuses) were used for genomic detection of *Brucella* spp. The PCR method described by (Leal-Klevezas *et al.,* 1995) targets a region within *omp2* gene. PCR detected amplicons of 193-bp in 6 (66.66%) samples (Fig. 1) of aborted fetuses and 68 (38.20%) sera samples (Fig. 2). Initially, whole blood samples were used as starting material for DNA extraction and none of the samples yielded a positive result, but, when respective sera samples were used, 68 samples showed bands (193 bp) specific for *Brucella* spp., indicating a likelihood of presence of host DNA that could affect the detection of *Brucella* spp. (Wang *et al.*, 2014) reported that presence of large amounts of host DNA caused a dramatic decrease in PCR sensitivity due to its competitive non-specific hybridization with primers and suggested that serum should be preferred over whole blood as starting material for DNA extraction.

PCR based assays have been developed for identification of the genus *Brucella* from cultures, animal/human tissues and animal products (Mahajan *et al.,* 2017). The method described by (Bricker and Halling, 1994) was used

for species differentiation. In all the six positive fetal stomach contents *B. abortus* was detected (Fig 3). However sera samples positive in *Brucella* genus PCR, failed to yield positive results in species specific PCR. This may be due to low concentration of the template DNA in the sample, implying that sera is not suitable starting material for *Brucella* species specific PCR. The causative agent of the abortion in all the six positive fetal stomach contents was *B. abortus* only and not *B. meltensis.* This was supported by the fact that the disease in cattle is predominantly caused by *B. abortus* and infrequently by *B. melitensis* (OIE, 2008; Khan and Zahoor, 2018).

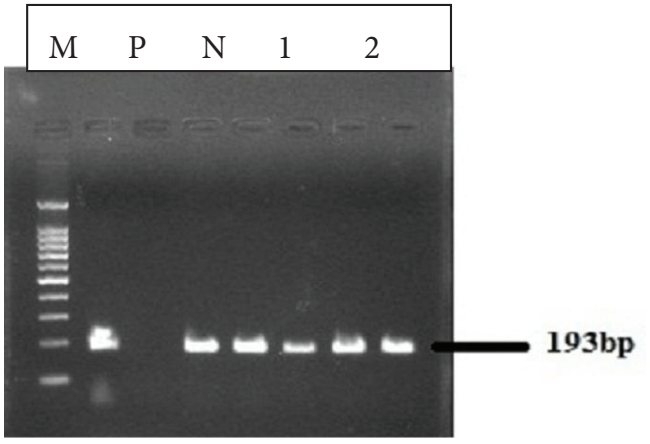

Fig. 1: Genus Specific PCR from aborted tissue samples
Lane M: Molecular weight marker
Lane P & N: Positive and Negative control
Lane 1-5: Samples

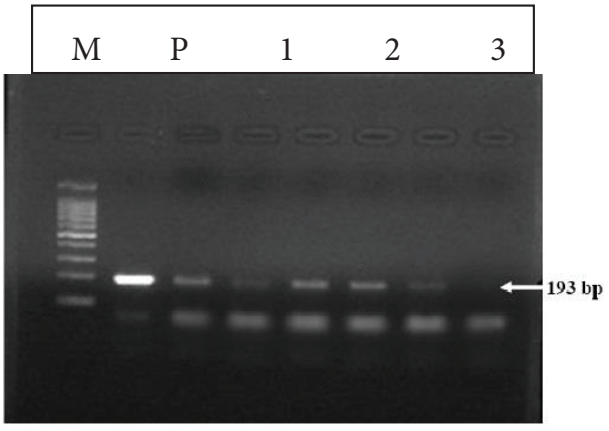

Fig. 2: Genus Specific PCR from aborted tissue samples
Lane M: Molecular weight marker
Lane P & N: Positive and Negative control
Lane 1-5: Samples

The method described by (Goni *et al.,* 2008) successfully differentiated *B. abortus* vaccine and field strains. This robust assay for the identification of *Brucella* is fast, safe and has high throughput. The practical utility of Bruce-ladder for typing purpose is evident, since some of the laborious and long lasting microbiological methods could be avoided (Goni *et al.,* 2008). The method works well irrespective of the cultural conditions, DNA extraction methods or thermo-cyclers used (Goni *et al.,* 2008). DNA from six *B. abortus* positive samples when subjected to Bruce ladder PCR amplified five fragments of 1682, 794, 587, 450 and 152 bp in size (Fig. 4). However, *B. abortus* S-19 DNA did not produce 587 bp fragment common to *Brucella* strains tested, enabling easy distinction of vaccine from the field strains. Bruce ladder PCR protocol described by (Goni *et al.,* 2008) is very simple and can identify and differentiate all known *Brucella* spp, including the *Brucella* isolates from marine animals in a single go. Above all it provides an easy and convenient way to differentiate the vaccine strain from the field strain and thereby, reducing the time for assay outcome and risk to laboratory workers.

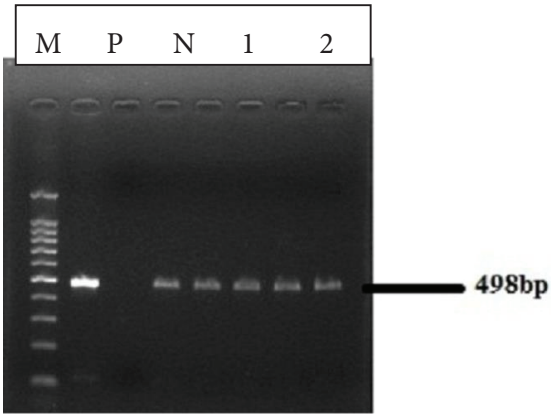

Fig. 3: *Brucella* species specific PCR
Lane M: Molecular weight marker
Lane P & N: Positive and Negative control
Lane 1-5: Samples

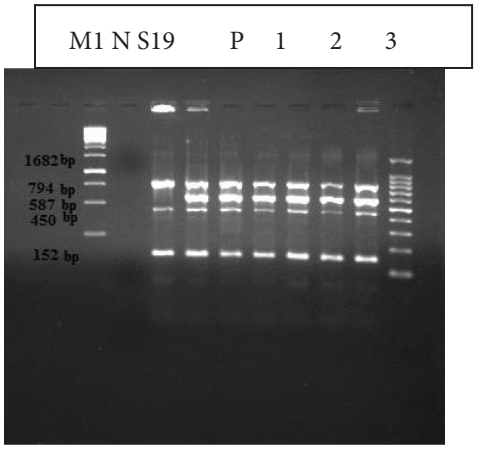

Fig. 4: PCR for differentiation of Field and Vaccine Strains
Lane M1 & M2: Molecular weight marker
Lane N, P & S19: Negative and Positive control, and B. abortus S19
Lane 1-5: Samples

Success of a vaccination programme depends on how best infected and vaccinated animals can be differentiated from each other. The Bruce ladder PCR can prove as an indispensable tool in differentiation of *Brucella* infected and vaccinated animals, thereby eliminating chances of categorizing sero-converts due to vaccination as sero-positive for brucellosis, as is seen in conventional sero-assays used for diagnosis of this disease in animals. This will augment the detection of infected animals especially in the incubatory phase and also those which fail to mount a detectable immune response (non-responders). As per (Islam *et al.,* 2014), the detection of *Brucellae* is complicated by chronic nature of infection and its asymptomatic course in heifers and young ones before its clinical manifestation. Moreover, it is quite challenging to establish at which time *Brucellae* may be present in the circulation due to obscure host pathogen interaction (Islam *et al.,* 2014). PCR in this regard can be an indispensible aid for prompt diagnosis of infection especially in heifers and young calves, before the organism can hide in its sites of predilection, which can be instrumental in bringing down the prevalence of this zoonotic disease to an economically justifiable level.

CONCLUSIONS

Farm bio-security measures aimed at keeping livestock free from brucellosis will play a crucial role in safeguarding human, animal and environmental health, which will subsequently help in disrupting the transmission cycle of this zoonosis. Its early and precise diagnosis in animals will serve in putting in place a suitable control strategy, which will help in eradication of this disease from animals. At the same time application of molecular tools like PCR will increase the likelihood of detecting the infected animals especially those, in the incubatory phase and differentiation of field from vaccine strains, compared to serological assays. This will help in devising a suitable surveillance mechanism for control and eradication of this disease.

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CONFLICT OF INTEREST

Authors have no relevant interests to disclose.

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