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BIOTECHNOLOGY

Diagnosis of Brucellosis using Molecular Techniques from Various Clinical Samples

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ABSTRACT

Brucellosis is an important zoonosis and a significant cause of reproductive losses in animals Abortion .In presence study 115 clinical samples were collected from cattle and buffalo, it all sample were analyzed with genus specific PCR *bcsp31gene*, those samples give positive result in genus PCR analyzed with species specific IS711gene PCR, 11 samples give positive result out of 115 samples in genus and species specific PCR. Highest percentage of positive result showed in Cotyledon (40%), Placenta (25%) and Vaginal discharge (20%) when the blood and Vaginal swab showed only 6% and 5.12% positive result in genus and species specific PCR, those all infected cattle and buffalo detected *Brucella abortus* positive. it means the cotyledon of aborted animal was more reliable clinical sample for molecular diagnosis of brucellosis in cattle and buffalo.

Highlights

- Brucellosis is an important zoonosis and a significant cause of reproductive losses in animals Abortion.
- In case of brucellosis Generally the cattle and buffalo infected with *Brucella abortus*.
- Cotyledon of aborted animal was more reliable clinical sample for molecular diagnosis of brucellosis in cattle and buffalo.

Keywords: Sample collection and preservation, DNA Extrection, detection of genus, detection of species, all sample *Brucella abortus* positive detected, highest percentage positive sample showed in cotyledon (40%).

Brucellosis is an important zoonosis and a significant cause of reproductive losses in animals Abortion. Placentitis, epididymitis and orchitis are the most common clinical manifestations (Patel *et al.*, 2015). In humans, brucellosis is a debilitating and chronic disease, which may affect a variety of organs. In view of the considerable problems related to direct diagnosis of brucellosis in animals and human, the present study envisaged the appraisal of detection of brucella genome in suspected clinical samples by bcsp 31genus PCR and LAMP assay (Ohtsuki *et al.*, 2008) followed by the identification and characterization of brucella species by species specified PCR, Bruce ladder multiplex PCR (Ester, *et al.*, 2014) and Real time PCR (Ying, *et al.*, 2014).

MATERIALS AND METHODS

A total of 115 clinical sample viz., Blood, Vaginal swab, Vaginal discharge, Placenta, Hygroma fluid, orchitis fluid, and cotyledon collected from Cattle and Buffalo (Table 1). Extraction of DNA by DNeasy blood and tissue kit as per protocol

(Qiagen), the extracted DNA processed for detection of genus with *bcsp31gene* for genus specified PCR (as per Table 2&3), Genus specific Positive sample processed for identification of species specified PCR IS711 (as per Table 2&3).

Table 1: Samples collected and detected positive for *Brucella abortus*

Types of sample	Number	Detected positive	Percentage of positive sample
Blood	50	3	6%
Vaginal swab	39	2	5.12%
Vaginal discharge	05	1	20%
Placenta	12	3	25%
Hygroma fluid	02	00	00
Orchitis fluid	02	00	00
Cotyledon	05	2	40%
Total	115	11	9.56%

Table 2: genus and species specific primers

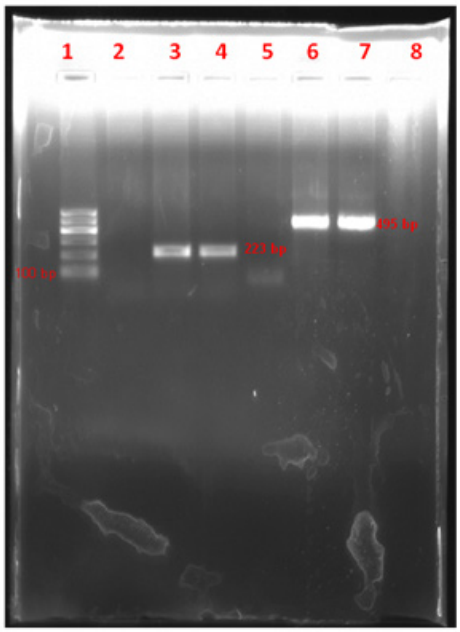
Genus specific primers:					
Sl. No.	**Primer**	**Forward/Reverse**	**Sequence (5′-3′)**	**Product size (bp)**	**Reference**
1	B4 (*BCSP31*)	Forward	TGG CTC GGT TGC CAA TAT CAA	**223** bp	Bailly *et al.* (1992)
2	B5 (*BCSP31*)	Reverse	CGC GCT TGC CTT TCA GGT CTG		
Species specific primers:					
Sl. No	**Primer**	**Forward/Reverse**	**Sequence (5′-3′)**	**Product size (bp)**	**Reference**
1	IS711 (*B. abortus*)	Forward	GACGAACGGAATTTTTCCAATCCC	498 bp	Bricker and Halling (1994).
2	IS711 (*B. abortus*)	Reverse	TGCCGATCACTTAAGGGCCTTCAT		

Table 3: Amount of PCR component and cycle condition for PCR

Sl. No.	Components	Aliquot(ul)
1	PCR Master Mix (2X)	12.5 µl
2	Forward Primer (10 pmol/µl)	1 µl
3	Reverse Primer (10 pmol/µl)	1 µl
4	Template DNA	2 µl
5	Nuclease free water	8.5 µl

Steps and conditions of thermal cycling for different primer pairs in PCR for B4/B5 and IS711

Steps	Temperature	Duration	Number of cycle
Initial Denaturation	93°C	5 min.	1 cycles
Denaturation	90°C	1 min.	
Annealing	64°C	30 sec.	35 cycles
Extension	72°C	1 min.	
Final extension	72°C	10 min.	1 cycles



1. Ladder
2. NTC
3. 223 bp genus positive (bcsp31)
4. 223 bp genus positive (bcsp31)
5. NTC
6. 495 bp (IS711)
7. 495 bp (IS711)
8. Sample- Negative

Fig. 1: Genus and Species specific PCR for Brucellosis

RESULTS AND DISCUSSION

Total 11 sample were detected positive in genus and species specific PCR out of 115 clinical sample (Fig. 1) and those all sample were *Brucella abortus* positive as per the result of species specific PCR (Table 1) but highest number of positive result showed by cotyledon (40%) placenta (25%) and vaginals discharge (20%) then blood and vaginals swab, it means that cotyledon of the aborted cattle and buffalo was more reliable than blood and vaginals swab for molecular diagnosis of brucellosis in cattle and buffalo. Hygroma fluid and orchitis fluid was not give only single positive result it means Hygroma and orchitis fluid was not reliable for molecular diagnosis of brucellosis in our research study.

On isolation of from samples, only one sample yielded *Brucella abortus*. The results showed that younger buffaloes were less infected than adults. Prevalence of Brucellosis was higher in Satwari block of Jammu province compared to other study area. The presence of Brucellosis in bovine may pose a significant economic loss to the farmer and a public health hazard to the general population (Malik *et al.*, 2014).

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Conclusion

The present study indicate the brucellosis prevalent in Gujarat, Major species prevalent is brucella abortus, the PCR technique was sensitive and specific in detection and identification of brucella from clinical sample and cotyledon was more reliable sample for diagnosis of brucellosis in cattle and buffalo

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