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Standardisation of loop-mediated isothermal amplification for the screening of bovine brucellosis

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Abstract
Brucellosis caused by *Brucella* spp. is one of the most widespread zoonotic diseases worldwide, with high impact on production and reproduction of animals and public health which in turn affects the economy of the nation. The present study aims to standardise loop-mediated isothermal amplification for the detection of *Brucella* in bovines in Kerala. The samples collected include uterine discharges after abortion, vaginal discharges from repeat breeders and aborted foetal internal organs. The primers based on IS*711* specific gene were used and subjected to loop-mediated isothermal amplification under optimal concentration of reagents, temperature and time. Twenty-one out of one hundred and one samples were positive revealing a green fluorescence under UV light. As loop-mediated isothermal amplification is a sensitive, specific, inexpensive and field oriented test, it can be exploited for the detection and management of the disease during its acute phase.

Keywords: Brucellosis, loop-mediated isothermal amplification, IS711gene, fluorescence, field oriented test

1. Introduction
Brucellosis is a zoonotic disease, caused by the bacteria of genus *Brucella* which are Gram-negative, aerobic, facultative intracellular coccobacilli, coming under class *α-proteobacteria* and family *Brucellaceae* (Pappas *et al.,* 2005) [8]. Bovine brucellosis is usually caused by *B. abortus*, less occasionally by *B. melitensis*, and *B. suis* (OIE, 2016) [5]. In bovines, transmission of disease mainly occurs by ingestion of contaminated material following an abortion and occasionally through aerosol, udder and conjunctiva (Hirsh *et al*., 2005; Corbel, 2006; OIE, 2016) [2, 1, 5]. The primary clinical signs include abortion in late trimester, retained foetal membranes and birth of weak calves (Roberts, 1999; Olsen *et al.,* 2004) [10, 6]. Severe economic loss in livestock industry and huge zoonotic impacts of this disease demands an early diagnosis to prevent its spread. A rapid antigen detection test at field level is necessary for the prevention and management of the disease. The present study was undertaken to standardise an antigen detection test, loop-mediated isothermal amplification (LAMP) for the detection of *Brucella* in bovines.

2. Materials and Methods

Table 1: Sequence of primers used for LAMP assay

Primers	Sequence
Forward outer primer (F3)	5’ GCCGATCACTTAAGGGCC 3’
Reverse outer primer (B3)	5’ CAATGTTTTCTCGCATCGCA 3’
Forward inner primer (FIP)	5’GGCTGTACAAGGAACGCCATCATTTTACGACGATAGCGTTTCAACT 3’
Reverse inner primer (BIP)	5’TTGTTGGGACACTGGAACGTGTTTTTTCATTGCCAGCCATCTCAAG 3’
Loop forward primer (Loop F)	5’GATTGAATGCTTTTTTAACA 3’
Loop reverse primer (Loop R)	5’CTTGGATCTGAGCCGTTG 3’

Table 2: Composition of a single reaction mix for LAMP

Components	Volume
Forward outer primer (10 p*M*/ µL)	0.5 µL
Backward outer primer (10 p*M*/ µL)	0.5 µL
Forward inner primer (10 p*M*/ µL)	0.5 µL
Backward inner primer (10 p*M*/ µL)	0.5 µL
Loop F (10 p*M*/ µL)	0.5 µL
Loop R (10 p*M*/ µL)	0.5 µL
Thermopol reaction buffer (10X)	2.5 µL
dNTPs	3.0 µL
$MgSO_4$ (25 mM)	2.0 µL
Betaine (5 M)	2.5 µL
Bst DNA Polymerase (8000 U/mL)	2.0 µL
Template DNA	4.0 µL
Nuclease free water	6.0 µL
Total volume	25 µL

A total of 101 clinical samples were collected from bovines, immediately transported to the laboratory, extracted DNA and stored at -20 ℃ until use. The samples included aborted foetal internal organs and stomach contents, uterine discharges from aborted dams and vaginal discharge from repeat breeder animals. The IS*711* gene sequence from *Brucella suis* 1330 (GenBank Accession no. AE014292.2) was selected as the target for designing six specific LAMP primers (Sancho *et al*., 2013) [11] which are depicted in table 1.

Primers were custom synthesised (Sigma-Aldrich, Bangalore) and obtained in lyophilised form. All the primers were reconstituted and working solution was made upto 10 pmol/ µL for further use. The DNA was extracted using HiPurA™ Multi-Sample DNA Purification Kit (HiMedia Laboratories Pvt. Ltd.) according to the manufacturer's guidelines. The concentrations of reagents were optimised in a total reaction volume of 25 µL. De-oxy Ribo Nucleic Acid extracted from *Brucella abortus* strain 19 vaccine, a commercial live vaccine was used for the preparation of positive control and nuclease free water served as negative control. The composition of the reaction mixture is given in table 2. The whole isothermal reaction was performed in a simple water bath by incubating the reaction mixture at 60 ℃ for 60 min.

3. Results and Discussion

Twenty-one out of one hundred and one samples were found to be positive for *Brucella*. Upon addition of 2µL of SYBR™ Safe DNA Gel Stain (Invitrogen™), positive samples developed a green fluorescence under UV light which is shown in figure 1. No fluorescence could be observed in negative samples. Positive samples were confirmed by formation of ladder-like pattern on agarose gel electrophoresis. Electrophoresis was carried out at 80 V for 30 minutes. The gel was visualised under UV transilluminator and the results were documented in a gel documentation system (Bio-Rad laboratories, USA).The gel image is depicted in figure 2.

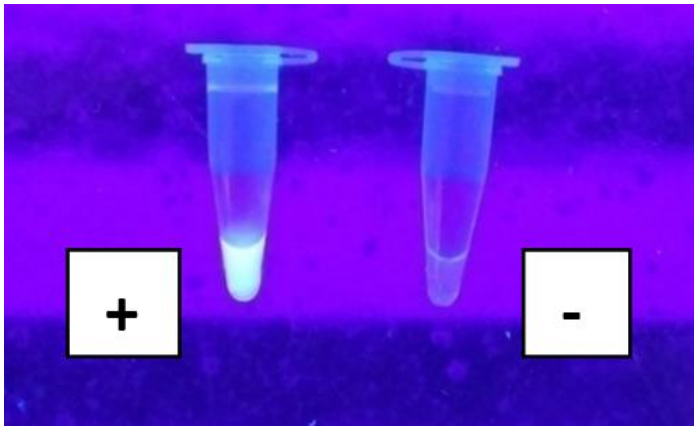

Fig 1: Positive sample with fluorescence (left) and negative sample without fluorescence (right) (representation)

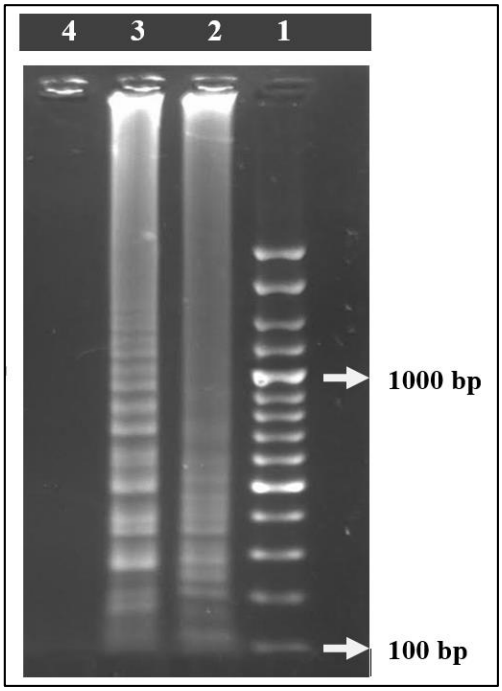

Fig 2: Agarose gel electrophoresis of LAMP products (representation)
Lane 1: 100 bp DNA ladder
Lanes 2: Positive control DNA
Lane 3: Positive clinical sample
Lane 4: Negative control DNA

The gold standard technique for diagnosis of brucellosis is isolation, but it is time consuming and need biosafety measures and skilled personnels (Lage *et al.,* 2008) [3]. Serological tests often result in false positive reaction because of the presence of antibodies due to vaccination and cross reactions with other Gram-negative bacteria (Poester *et al.,* 2010) [9]. This study suggested that LAMP could be used as an effective molecular technique for the detection of *Brucella* in bovines as it is rapid, sensitive, specific and cost effective (Ohtsuki *et al.,* 2008; Pan *et al.,* 2011; Sancho *et al.,* 2013) [4, 7, 11].

4. Conclusion

Prevention and control of brucellosis is of prime importance, as there is no human vaccines currently available and isolation and slaughtering are the preventive measures in animals. Loop-mediated isothermal amplification test is very convenient as it does not require any sophisticated equipments for the detection of causative agent. It is a highly sensitive and specific diagnostic technique as it detects antigens at the acute phase of the disease, so that effective and appropriate management of the disease could be undertaken.

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6. References

1. Corbel MJ. Brucellosis in humans and animals. World Health Organization, Geneva. 2006, 89.
2. Hirsh DC, MacLachlan NJ, Walker RL. Veterinary Microbiology. (2nd Ed.). Blackwell Pub., 2005, 536.
3. Lage AP, Poester FP, Paixao TA, Silva TMA, Xavier MN, Minharro S *et al.* Brucelose bovina: uma atualizaçao. Revista Brasileira de Reproduçao Animal. 2008; 32(3):202-212.
4. Ohtsuki R, Kawamoto K, Kato Y, Shah MM, Ezaki T, Makino SI. Rapid detection of Brucella spp. by the loop-mediated isothermal amplification method. Journal of Applied Microbiology. 2008; 104(6):1815-1823.
5. OIE [World Organisation for Animal Health]. Brucellosis (*Brucella abortus*, *B. melitensis* and *B. suis*) (Infection with *B. abortus*, *B. melitensis* and *B. suis*). In: OIE Terrestrial Manual. World Organisation for Animal Health, Paris, France, 2016, 616-650.
6. Olsen JE, Brown DJ, Thomsen LE, Platt DJ, Chadfield MS. Differences in the carriage and the ability to utilize the serotype associated virulence plasmid in strains of *Salmonella enterica* serotype Typhimurium investigated by use of a self-transferable virulence plasmid, pOG669. Microbial Pathogenesis. 2004; 36(6):337-347.
7. Pan W, Shen HY, Zhao MQ, Tu CM, Dong XY, Yi L, Wang Y, Chen D. Development and application of the novel visual loop-mediated isothermal amplification of omp 25 sequence for rapid detection of *Brucella* sp. Journal of Animal Veterinary advances. 2011; 10(16):2120-2126.
8. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. The New England Journal of Medicine. 2005; 352(22):2325-2336.
9. Poester FP, Nielsen K, Samartino LE, Yu LW. Diagnosis of brucellosis. The Open Veterinary Science Journal. 2010; 4(1):46-60.
10. Roberts SJ. Veterinary Obstetrics and Genital Diseases (Theriogenology). (2nd Ed.). CBS Publishers and Distributors Pvt. Ltd., New Delhi, India, 1999, 776.
11. Sancho MP, Seco TG, Arrogante L, Garcia N, Martinez I, Guerrier AD, Perales A, Goyache J, Dominguez L, Alvarez J. Development and evaluation of an IS711-based loop mediated isothermal amplification method (LAMP) for detection of *Brucella* spp. on clinical samples. Research in Veterinary Science. 2013; 95(2):489-494.