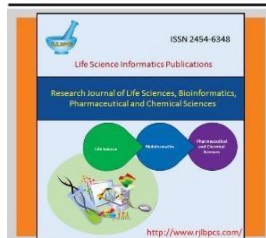
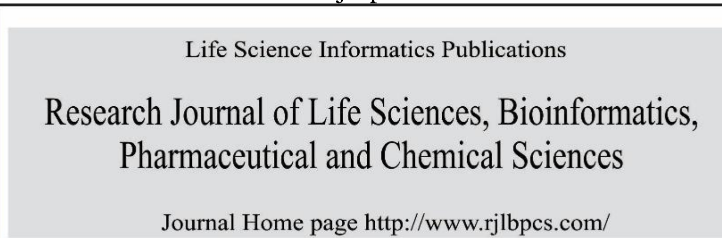
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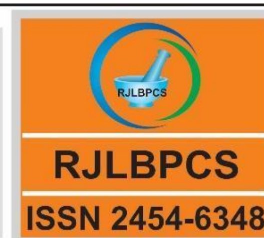

Original Research Article **DOI: 10.26479/2022.0802.04**

EVALUATING SEROLOGICAL AND MOLECULAR METHODS USED FOR THE DETECTION OF BRUCELLA FROM CATTLE AND BUFFALO

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ABSTRACT: *B. abortus* and *B. mellitus* belong to the bacterial genus Brucella that has been infecting cattle and buffalo and is highly contagious. Various species of brucella also infect sheep, goats and dogs; and transmit to humans also. Early detection is possible using techniques like serological and molecular tests that also help eradicate the disease. Rose Bengal Plate Test, indirect ELISA and milk ring test are common and popular serological testing methods and are used in the present study along with Conventional PCR, TaqMan Probe-based and SYBR green dye-based PCR assay. A total of 1154 samples including Blood, vaginal swab, placental fluid, foetus, vaginal discharge, orchitis fluid and hygroma fluid are used for serological and molecular testing. Among 252 total serologically tested buffalos 19 were positive for RBPT (19/203), 17 were positive for iELISA (17/203) and 47 were positive for MRT (47/252) and among 902 total serologically tested cattle 201 were RBPT positive (201/902), 197 were iELISA positive (197/902) and 69 were MRT positive (69/167) while only 6 samples were positive for PCR (6/353). The results concluded that the combination of serological and molecular testing increases the specificity, accuracy, speed and reducibility of testing.

Keywords: Molecular analysis, Brucella, Seroprevalence, PCR.

Article History: Received: April 10, 2022; Revised: April 22, 2022; Accepted: April 30, 2022.

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1.INTRODUCTION

B.abortus and *B. Mellitus* belong to the bacterial genus Brucella that has been infecting cattle and buffalo and is highly contagious. Various species of brucella also infect sheep, goats and dogs; and transmit to humans also [1][2]. The infection commonly known as brucellosis is hence zoonotic with serious health implications. In India, the condition is endemic and a major health concern. Common symptoms of the present conditions are stillbirth, infertility, placentitis, epididymitis and

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orchitis [3]. Serious clinical manifestations are also reported in humans who mainly acquire the disease by consumption of unpasteurized milk and direct contact with infected livestock, however, laboratory personnel may also get the disease during testing and culturing [4]. Early detection is possible using techniques like serological and molecular tests that also help eradicate the disease. Rose Bengal Plate Test, indirect ELISA and milk ring test are common and popular serological testing methods in which largely, neutralizing antibodies are allowed to cross-react with the sample antigen if present [5]. These tests are popular, simple, cost-effective and rapid methods used for investigating infected herds [6]. However, the serological results are inappropriate as other antigenically related bacteria may also cross-react [7] and also can not discriminate against infected and exposed animals [8]. Bacteriological culture, though considered a gold-standard method for detection of brucella, the technique is time-consuming, laborious and can infect the personnel. Furthermore, it is prone to contamination and requires various growth media supplements. Molecular testing, on the other side, such as conventional gel-based PCR assay, RT-PCR or multiplex Bruce ladder PCR comes with high sensitivity, specificity and accuracy. Besides, each molecular technique is safe to perform, reproducible, can detect as low as a single pathogen from the sample and provides results within 2 to 3 hours [9]. The present study has been designed to determine the importance of various techniques used in the detection, screening, identification and diagnosis of brucella and brucellosis.

2. MATERIALS AND METHODS

Sample collection

A total of 1154 samples including Blood, vaginal swab, placental fluid, foetus, vaginal discharge, orchitis fluid and hygroma fluid of buffalo (203) and cattle (902) were collected. None of the animals included in the present study were vaccinated. Each type of sample was collected and maintained as per the standard sample collection guidelines. Samples were processed for RBPT, iELISA, MRT, PCR and RT-PCR. The Rose Bengal Plate Test was performed using the manufacturer's protocol provided by the Institute of Animal Health, Hebbal (IAH) and Veterinary Biological, Banglore (VB). The iELISA test was performed as per the kit and instructions provided by the National Institute of Veterinary Epidemiology and Disease informatics, Banglore (NIVEDI). Percent positivity was taken into consideration at OD 445nm for interpreting results showing that the PP value >65 and between 55- 65 is manifested as strong and moderate positive, respectively while <55 is manifested as negative. The milk ring test was performed using the protocol provided by the kit manufacturer (RINGBIO). All serological tests were performed as per the instruction of the kit provider and protocols mentioned by Patel K *et al.,* 2007. For molecular analysis, DNA was extracted using the DNeasy Blood and tissue kit provided by Qiagen, USA to perform PCR and RT-PCR. *Brucella* genus-specific primers B4/B5 and *Brucellosis abortus* species-specific primers

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IS711 were used for conventional PCR using a standard protocol provided in the literature. Real-time quantitative PCR was performed using the TaqMan probe and SYBR green dye-based techniques. All the protocols, reagent preparation, primer sequences, PCR cycling conditions and other information is given and used from the research paper of Patel K *et al.,* 2018 & Patel B *et al.,* 2017 [10][11].

3. RESULTS AND DISCUSSION

A total of 1154, buffalo (252) and cattle (902), and 353 buffalo (146) and cattle (207) samples were processed for serological testing and molecular analysis, respectively. All 252 buffalo samples were tested by RBPT and MRT while 203 samples were tested by iELISA; on the other hand, all 902 cattle samples were tested for RBPT and iELISA and 167 samples were tested for MRT. Notedly, for molecular analysis, all the 353 samples were processed by all three techniques. Among 252 total serologically tested buffalos 19 were positive for RBPT (19/203), 17 were positive for iELISA (17/203) and 47 were positive for MRT (47/252). RBPT, iELISA and MRT positivity rate is 9.36%, 8.37% and 18.65%, respectively. Among 902 total serologically tested cattle 201 were RBPT positive (201/902), 197 were iELISA positive (197/902) and 69 were MRT positive (69/167) with the positivity rate of 22.28%, 21.84% and 41.32%, respectively. Between 1154 total tested herds 220, 214 and 116 were positive for RBPT, iELISA and MRT. Molecular analysis using PCR and RT-PCR was performed on 146 buffalo and 207 cattle samples using the set of BCPC31 brucella genus-specific primers (B4/B5) & IS711 species-specific primers for conventional PCR, SYBR green dye and probe-based real-time technique. 6 samples were positive for all three techniques among which 4 were buffalo and 2 were cattle DNA with the positivity rate of 2.73% and 0.96%. The results are given in Table 1.

Table 1. Serological testing of various buffalo and cattle samples

	RBPT test		**iELISA Test**		**MRT test**		**Molecular testing**	
	RBPT tested	RBPT positive	iELISA tested	iELISA positive	MRT tested	MRT positive	PCR Total tested	PCR positive
Buffalo	252	19 (9.36%)	203	17 (8.37%)	252	47(18.65%)	146	4(2.73%)
Cattle	902	201 (22.28%)	902	197 (21.84%)	167	69 (41.32%)	207	2 (0.96%)
Total	**1154**	**220 (19.06%)**	**1105**	**214(19.36 %)**	**419**	**116(27.68%)**	**353**	**6 (1.69%)**

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Table 2. Molecular testing using conventional PCR, Probe-based RT-PCR and SYBR green dye-based RT PCR.

	Molecular genetic technique				
	Total samples	**Conventional PCR**	**SYBR green RT-PCR**	**Probe-based RT-PCR**	**Positivity rate (%)**
Buffalo	146	4	4	4	2.73%
Cattle	207	2	2	2	0.96%
Total	**353**	6	6	6	**1.69%**

A total of 6 positive samples among 253 samples tested using various PCR variants showed amplification for both genus-specific primers (bcsp31) and species-specific primers (IS711) yielding 223 and 498bp fragments on 2% standard agarose gel electrophoresis and indicated that samples have B. *abortus*. Two different real-time quantification assays also provided the same results using the same type of primer sets and validating the fact that the brucellosis that occurred in cattle and buffalo was by the *Brucella abortus* Note, the results of quantification were evaluated using techniques like melting curve analysis and Ct value. Serological testing along with molecular analysis increases the efficiency, accuracy and specificity of the study by many folds, however, serological tests are used for surveillance purposes only as they can cross-react with other infections too [7]. On the other hand, molecular tests like conventional PCR, real-time PCR or DNA sequencing have been known for their accuracy, sensitivity, reproducibility and speed [12]. Interestingly, the combination of tests of these two groups increases testing feasibility, greatly hence, sero-tests like RBT, iELISA, milk ring test and molecular tests like PCR and RT-PCR were used in the present study. The culture was, however, considered the gold standard method but is tedious, time-consuming and contamination prone [13] [14] and so is not included in the present study [15].

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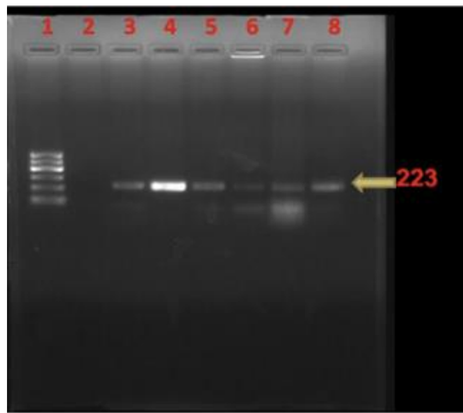

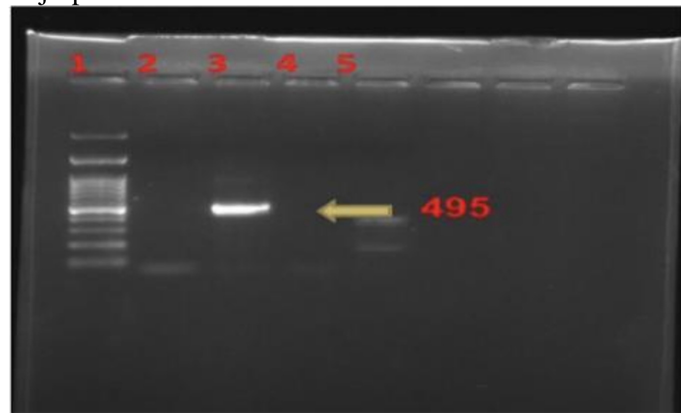

(A) (B)

Image 1: (A) Agarose gel electrophoresis results of PCR using B4/B5 genus-specific primer. (G) Agarose gel electrophoresis results of PCR using the IS711 species-specific primers.

The results of the present study Manifested the highest rate of brucellosis in cattle using the serological tests while lower by PCR testing. The milk ring test showed the highest positivity rate of 27.68% for a total number of samples and 18.65% and 41.32% for buffalo and cattle individually followed by iELISA and RBPT whereas other previous studies showed Rose Bengal Test with the highest positivity rate [16]. Interestingly, The highest and lowest seroprevalence using iELISA is reported in two studies [15][17] which were 82.6% and 7.3%, respectively. And postulated that none of the serological testing techniques are consistent. other two research studies described that the Rose Bengal test is sensitive enough to find brucellosis but has lower specificity and can cross-react with other pathogenic antigens [2][18]. In the present study, the seroprevalence of cattle is very high in comparison to the buffalo, the results are supported by various previous studies [16] and describe that the higher prevalence may be attributed to species specificity. In our observations, the inconsistency in the number of samples may be another factor resulting in higher seroprevalence in cattle. As described in the previous studies, PCR-based assays like the conventional PCR amplification or species-specific real-time amplification for the detection of Brucella species are extremely sensitive techniques with higher specificity as well. Molecular techniques are rapid, robust and reproducible [19]. Out of 146 and 207 total buffalo and cattle tested for PCR and RT-PCR, only 4 and 2 samples gave positive results, respectively. Previous reports expanded the present knowledge and explained that PCR remained a “top-notch” technique among all the testing methods. DNA extraction protocol is one of the important laboratory procedures for getting excellency in PCR. Though a ready-to-use DNA extraction kit was used in the present study, Poor DNA extracts, low quantity of DNA samples and inadequate sample collection are several factors that lead to non-amplification, reaction failure or negative results in the present study therefore out of 353 total samples processed the positivity rate by any PCR technique is 1.69% only. In a study, 5 different DNA extraction protocols, including the kit-based assay, were performed and achieved the PCR

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positivity rate of 55% for molecular analysis which concluded that DNA extraction has significant importance in the molecular analysis [19].

4. CONCLUSION

In conclusion, molecular analysis such as various types of PCR assays are reproducible and accurate and can elevate the screening and diagnostic procedure. Nonetheless, serological tests are cheap and rapid detection methods and useful ones for the primary screening of brucellosis. The combination of these two techniques is highly recommended for brucellosis screening in animals. In addition, our study also demonstrated that the quality and quantity of DNA are directly proportional to the PCR positivity rate so a good DNA extraction method should be considered an important criterion for testing.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

FUNDING

None.

ACKNOWLEDGEMENT

We are thankful to Dr Tushar Chauhan from Genetic Education (www.geneticeducation.co.in) for providing guidance and valuable suggestions to write this research article.

CONFLICT OF INTEREST

The author declared no conflicts of interest.

ETHICAL APPROVAL

The present study has been approved by the ethical committee of Sankalchand University. All the procedures are followed using the standard guidelines provided.

REFERENCES

1. Godfroid J, Scholz HC, Barbier T, Nicolas C, Wattiau P, Fretin D, Whatmore AM, Cloeckaert A, Blasco JM, Moriyon I, Saegerman C, Muma JB, Al Dahouk S, Neubauer H, Letesson JJ. Brucellosis at the animal/ecosystem/human interface at the beginning of the 21st century. Prev Vet Med. 2011 Nov 1;102(2):118-31.
2. Corbel M. Comparison of Brucella abortus and B. melitensis antigens for the Rose Bengal plate test on sera from cattle infected with B. abortus biovar-5. Vet. Rec. 1995; 117(15): 385-386.

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3. McDermott J, & Arimi S. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. Veterinary microbiology 2002, 90; 1(4); 111-134.
4. Wallach JC, Samartino LE, Efron A, Baldi PC. Human infection by *Brucella melitensis*: an outbreak attributed to contact with infected goats, FEMS Immunology & Medical Microbiology, Volume 19, Issue 4, December 1997, Pages 315–321.
5. Alton G, Jones L, Angus R, Verger J. techniques for the brucellosis laboratory. 1st edition. Paris: Institut National De la Recherche Ergonomique 1988.
6. Noriello S. Laboratory-acquired brucellosis. Emerging Infectious Disease 2004; 10: 1848-1850.
7. Díaz-Aparicio E, Marín C, Alonso-Urmeneta B, Aragón V, Pérez-Ortiz S, Pardo M, Blasco JM, Díaz R, Moriyón I. Evaluation of serological tests for diagnosis of Brucella melitensis infection of goats. J Clin Microbiol. 1994 May;32(5):1159-65.
8. Borriello G, Capparelli R, Bianco M, Fenizia D, Alfano F, Capuano F, Ercolini D, Parisi A, Roperto S, Iannelli D. Genetic resistance to Brucella abortus in the water buffalo (Bubalus bubalis). Infect Immun. 2006 Apr;74(4):2115-20.
9. Garibyan L, Avashia N. Polymerase chain reaction. J Invest Dermatol. 2013 Mar;133(3):1-4.
10. Patel K, Chauhan H, Patel S, Patel B, Shrimali M, Chandel B. Molecular detection of Brucella Abortus using BSCP31 and IS711 Gene-based PCR assay in cattle and buffalo. Buffalo Bulletin 2018; 37 (1), 71-80.
11. Patel B, Patel K, Patel S, Modi A, Chauhan H, Shrimali M, Dodiya V, Chandel B. Diagnosis of Brucellosis using Molecular Techniques from various Clinical Samples. International Journal of Agriculture, Environment and Biotechnology 2017; 10(2), 267-270.
12. Zerva L, Bourantas K, Mitka S, Kansouzidou A, Legakis NJ. Serum is the preferred clinical specimen for diagnosis of human brucellosis by PCR. J Clin Microbiol. 2001 Apr;39(4):1661-4.
13. Cloeckaert A, Verger JM, Grayon M, Vizcaino N. Molecular and immunological characterization of the major outer membrane proteins of Brucella. FEMS Microbiology Letters 1996; 145, 1-8.
14. Refai M. Applications of biotechnology in the diagnosis and control of brucellosis in the Near East Region. World J Microbiol Biotechnol. 2003; 19, 443-449.
15. Saadat S, Mardaneh J, Ahouran M, Mohammadzadeh A, Ardebili A, Yousefi M, Mansouri M. Diagnosis of Cattle Brucellosis by PCR and serological Methods: Comparison of Diagnostic Tests. Biomed Pharmacol J 2007; 10(2), 881-88.
16. Ghazi I. and Al-Shemmari, M. Comparative study between conventional and molecular tests to detect the incidence of brucellosis in Cattle and Buffaloes in Babylon and Karbala provinces. Scientific Journal of Medical Research 2018, 2(5), 7-13.
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17. Elsheikh A, Masoud E, Mostafa M, Elkhawankya M. Seroprevalence of 2 zoonotic diseases in southwestern Saudi Arabia: Rift valley fever and Brucellosis. Sudi Med J. 2011; 32: 740-41.
18. Abubakar M, Mansoor M, Arshed M. Bovine Brucellosis: Old and new concepts with Pakistan perspective. Pak. Vet. J. 2012, 32, 147-155.
19. Marianelli C, Martucciello A, Tarantino M, Vecchio R, Iovane G, Galiero G. Evaluation of molecular methods for the detection of Brucella species in water buffalo milk. J Dairy Sci. 2008 Oct;91(10):3779-86.

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