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Prevalence Studies on Brucellosis in Buffalo Population in Nearby Region of Hyderabad, Telangana

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ABSTRACT

Background: Brucellosis in India is highly endemic in many states and reported as the most prevalent disease in dairy cattle. Brucellosis is a highly contagious bacterial disease caused by various *Brucella* species mainly affecting wide range of different species of animals like cattle, sheep, goat and pigs. The present study was undertaken to ascertain the prevalence of brucellosis in suspected buffalo population showing reproductive disorders in selected dairy farms residing nearby region of Hyderabad, Telangana.

Methods: Total number of 204 vaginal swab samples were collected aseptically from suspected buffaloes in nearby private dairy farms and were screened by employing various diagnostic methods. Enzyme Linked Immuno Sorbent Assay (ELISA), Lateral Flow Assay (LFA) and molecular identification through Polymerase Chain Reaction (PCR) were conducted. The sensitivity of tests was recorded considering PCR as gold standard method.

Result: Screening of 204 vaginal swab samples and prevalence of brucellosis were recorded by ensuring the positive reactions and also depicting the sensitivity of various diagnostic tests namely ELISA, LFA and PCR accounting 92(45.1%), 87 (42.64%) and 113(55.4%) samples respectively. The specificity of both LFA and ELISA were recorded 100% when compared to PCR assay.

Key words: Brucellosis, ELISA, Lateral flow assay, PCR.

INTRODUCTION

Brucellosis is highly contagious zoonotic infection with economic significance, accounting approximately $3.4 billion annual losses in India (Singh, 2015). The *Brucella* organisms are gram negative, facultative, and intracellular bacteria significantly affecting domestic, wild animals and human population causing the infectious disease called brucellosis (Mathur, 1971). In animals, brucellosis widely affects reproductive system and mostly accompanied with fertility disorders, reduces the survivability of newborn calves and diminishes milk yield while in human the symptoms of disease are joint and muscle pain, headache, undulant fever, hepatomegaly, splenomegaly, night sweats and chills, marked asthenia and anorexia (Hugh-Jones, 2000). The illness has a significant negative socioeconomic impact, particularly in rural areas where the main sources of income are cattle rearing, milk production and preparation of dairy products. Despite wide usages of various serodiagnosis tests like Rose Bengal precipitation Test (RBPT), Lateral Flow Technology (LFT), Enzyme Linked Immunosorbent Assay (ELISA). These tests were used to detect Brucella antibodies in serum/blood in diagnosing the brucellosis, disease is still widespread and becoming more common in many poor nations as these tests are widely preferred since, its on par less costly and user friendly . Animals sometimes may be seronegative but can act as consistent source infection affecting healthy flocks. Specificity and sensitivity of tests play s important role to diagnose higher percentage of positive reaction. Numerous diagnostic methods, including molecular assays (PCR), serological (RBPT, Agar Gel Immunodiffusion Test (AGID), ELISA, LFT *etc*.) and cultural methods are available to determine the prevalence of brucellosis. PCR technique is highly precise, sensitive and targeting two major genetic targets BCSP31 and 16S – 23S r RNA it needs sophisticated laboratory facility and equipment. Sandwich ELISA will be more specific in their action by employing primary and secondary antibodies. Lateral flow assay is a rapid diagnostic method usually based upon detection of IgM or IgG antibodies, which was modified in the present study to

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detect brucella antigen in the clinical samples as pen side diagnostic kit, highly applicable to field conditions. In the present study, sensitivity, reliability and specificity of various diagnostic tests has been analysed, based upon PCR as gold standard technique.

MATERIALS AND MEHTODS

Collection of samples

The experimental study was undertaken for 12 months and collected the vaginal swabs from suspected buffalo population from the end of May 2019 to June 2020 from the nearby private farms in Hyderabad region. The collected samples were processed in the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, PVNRTVU, Rajendranagar, Hyderabad, Telangana, India.

All samples were processed according to the guidelines of WOAH (2023). The samples were collected carefully and cross-contamination was avoided during collection and transportation. The vaginal secretions from suspected buffaloes were collected using sterile swabs. The swabs were inserted into the vagina and once secretions adhered, the swab was removed and placed in a sterile tube. Each swab tube was aseptically packed, properly labeled, transported to laboratory and processed as early as and can be preserved for 2 hours at 4°C. Vaginal swabs were collected from 204 buffaloes from Kachiguda slaughterhouse and private dairy farms located in and around the Greater Hyderabad Municipal Corporation.

Detection of *Brucella* organisms in vaginal swab samples

Sandwich ELISA

Sandwich ELISA test procedure was followed with slight modification recommended by Hans *et al.* (2020). Poultry antibodies (IgY) and rabbit IgG antibodies used as primary and secondary antibodies. The vaginal swab (field) samples were added to wells and kept at 37°C for 45 minutes in incubator. Finally, 100 µl of stop solution was added and OD values were recorded at 605 nm wavelength in UV Spectrophotometry.

Lateral flow Assay

The lateral flow assay kit has been prepared and subjected for usage after standardization of conjugation process of polyclonal antibodies raised in rabbit with gold nanoparticles to enhance the specificity of test. The conjugate of Gold nano particle -Poly clonal antibody (rabbit) was sprayed on conjugation pad, poultry antibodies (IgY) as primary antibody were adhered at test line and antirabbit antibodies were adhered at control line on nitrocellulose membrane. The sample pad and absorbant pad were flanked at the ends of strip followed by conjugation pad, test line and control line which ensured single flow of direction of sample was supported by Poly Vinyl Chloride (PVC) material placed in plastic cassettes which serves as kit for diagnosis of brucellosis. The collected Vaginal swab samples were immersed in 2ml of sterile distilled water and properly mixed and 0.5ml of this mixture is placed on the sample pad of kit, after 2-5 minutes the development of red colour at control and test line were indicative for a positive result.

PCR assay

The PCR assay considered as referral test for brucellosis where the vaginal samples were processed and centrifuged at 2,348× g for 5 minutes. Supernatant was discarded, and the pellet was resuspended in 100 µl of nuclease free water and placed in a boiling water bath for 10 minutes. After heat treatment, the cell lysate was immediately kept on ice for 10 minutes followed by centrifugation at 2,348×g for 5 minutes. The supernatant containing the DNA was aliquoted into a sterile tube and stored at -20°C until further use (Vijaya Kumar, 2015).

A set of (B4/B5) primers derived from *Bcsp31* genes (Bailey *et al.,*1992) synthesized by Saha Gene, Hyderabad was used for PCR amplification of *Brucella* organism up to genus level shown in Table 1 and Primers were dissolved in nuclease-free water to obtain a concentration of 100pmol / µl (stock solution) and it was further diluted to obtain 10 picomoles/µl as working Solution.

Master mix was prepared by adding DNTP buffer@ 1.25 units (containing 50-200 µmole DNPT's,1-2 mM Magnesium chloride, 2.5 µl 10X assay buffer for Taq polymerase), forward and reverse primers to Milli Q water and in one vial positive control (heat killed vaccine) and in another vial no vaccine was added (negative control). DNTP's are added at 1.25 units (volume 10 µl) per vial. Primers are added in an amount of 2 picomoles (Volume 2 µl) per vial. Template was added (concentration 10^9CFU/2ml) @ 2µl to positive control vials. in negative control no template was added and final volume was made up to 20µl for both positive and negative control. The optimization and standardization of PCR steps as per conditions mentioned in Table 2.

Visualization of PCR products by agarose gel electrophoresis

To confirm the targeted PCR amplification, five µl of PCR products per tube were mixed with one µl of loading buffer and electrophoresed along with DNA molecular weight marker (Gene Ruler, MBI Fermantas). On a 1% agarose gel containing ethidium bromide (0.5 µg/ml) at constant

Table 1: Details of primers used in PCR for *Brucella* species detection.

Name of the primer		Gene code for protein/ Size	Base pairs	5' to 3' Sequence	Reference
F	B4 (F)	Bcsp31, 31 kD	223 bp	TGG CTC GGT TGC CAA TAT CAA	Baily *et al.* (1992)
R	B5 (F)	Bcsp31 ,31 kD		CGC GCT TGC CTT TCA GGT CTG	

50mV for 45 minutes in 0.5X TBE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system (Bio-Rad Gel Documentation and Imaging System, USA).

RESULTS AND DISCUSSION

The study was carried out on 204 suspected buffalo vaginal swab samples and various diagnostic methods were adopted and percentage of positive and negative were displayed in Table 3. Out of 204 samples, the results on sensitivity of ELISA were on higher side 45.1% with 92 samples shown positive reaction. Almost similar findings were reported with a prevalence of 43%, 44% and 46.6% as reported by Raheela Akhtar *et al.* (2010), Chauhan *et al.* (2000) and Jain *et al.* (2013) respectively. Lower prevalence of 19.4% (Abdel Hamid *et al.,* 2008), 18.20% (Sarkar *et al.,* 1987), 10.42% (Zahid *et al.,* 2002), 9.58% (Hussain *et al.*, 2008),8.15% (Patel, 2007) and 5.67% (Kanani, 2007). 3.5 % (Samaha *et al.,* 2008), 2.4% (Lodhi *et al.,* 1995) 1.9% (Rahman *et al.,* 2019) and 1.1% (Nawal and Ahmed 2008) were reported than the prevalence in the present study. Very high prevalence of 88.9% was reported by Kang'ethe *et al.* (2000). Nasir *et al.* (2014) observed higher prevalence (18.53%) in private dairy farms compared to government farms (14.70%) using ELISA. The high prevalence of brucellosis in buffaloes from private farms in the present study might be due to frequent introduction of new high yielding animals without proper serological tests and high prevalence of abortions (Nasir *et al.,* 2004).

LFA shown lesser 42.64% of positive reaction accounting 87 samples. Abdoel *et al.* (2008) reported very high prevalence of 90% in bovines using the kit developed by him. Shome *et al.* (2018) developed lateral flow assay for diagnosis of brucellosis and reported 6.6% prevalence which was less than the prevalence observed in present study.

PCR considered as preferred tool for definitive diagnosis for brucella DNA (Ulu-Kilic *et al*., 2013) similarly, PCR shown higher side with 113 samples positive reaction accounting 55.4% of positive reaction in the present study. Lower prevalence of 28.3%, 35.8% and 34.3% in buffaloes using vaginal swabs, blood, milk samples respectively were reported by AL-Shemmari (2018) by PCR assay. Very high prevalence of 87.5% using vaginal samples was reported by Romero *et.al*., (1995) compared to prevalence in the present study. A very low prevalence of 2.73% in buffaloes was reported by Patel *et al.* (2022).

Sensitivity and specificity of ELISA and LFA compared with PCR assay

The sensitivity and specificity of the tests on comparison with PCR as referral test was shown on Table 4. Out of 204 vaginal samples PCR assay has given 113 Positives whereas LFA and ELISA has given 92 and 87 positives. All the samples positive by the LFA and ELISA were positive by PCR. The sensitivity of the LFA and ELISA compared to PCR assay was 77 and 81.42% respectively. By LFA and ELISA 117 and 112 samples were negative whereas 91 samples were negative by PCR assay. All the PCR assay negatives

Table 2: Steps and conditions of PCR.

Step	Time	Temperature	Number of cycles
Initial denaturation	5 minutes	95°C	
Denaturation	30 seconds	95°C	
Annealing	30 seconds	46°C	35
Elongation	1 minute	72°C	
Final Elongation	5 minutes	72°C	

Table 3: Prevalence of Brucellosis recorded by different diagnostic methods.

Type of tests	No of samples	Positive samples	Percentage of positive samples	Negative samples	Percentage of negative samples
PCR	204	113	55.4%	91	44.6%
ELISA	204	92	45.1%	112	54.9%
LFT	204	87	42.64%	117	57.36%

Table 4: Sensitivity and specificity of ELISA and LFA compared with PCR assay.

Type of assay	Number of positive samples	Number of negative samples	Sensitivity of ELISA based on PCR	Specificity of ELISA based on PCR	Sensitivity of LFA based on PCR	Specificity of LFA based on PCR
PCR Assay	113	91	NA	NA	NA	NA
ELISA	92	112	92/113= 81.42 %	91/91= 100%	NA	NA
LFA	87	117	NA	NA	87/113 = 77 %	91/91 = 100%

were negative by ELISA and LFA assay. Hence the specificity of both LFA and ELISA was 100%.

CONCLUSION

Brucellosis is one of the important zoonotic infection that has to be diagnosed as early as possible since it has huge impact on productivity of the animal and also affecting the human population severely. For detection of Brucellosis there are so many reliable techniques available like PCR, ELISA and AGID *etc*, but reliability and efficiency has to be graded on basis of most reliable technique *i.e* PCR. In the present study, the prevalence of brucellosis in the Hyderabad region were analysed by employing various serological test like ELISA and LFA. Based upon the sensitivity and specificity the techniques were evaluated with gold standard technique on PCR. Out of 204 vaginal swabs tested for PCR assay, ELISA and LFA based 113, 92 and 87 samples were positive for *Brucella* accounting 55.4%, 45.1% and 42.64% prevalence respectively. The sensitivity of LFA and ELISA were 76.99% and 81.4% respectively whereas specificity was 100% for both tests.

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Conflict of interest

Authors have no competing interests that are directly or indirectly related to the work submitted for publication.

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