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Serological and Molecular Screening of Camels for Brucellosis in Bikaner, Rajasthan, India

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Authors' contribution

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Brucellosis is an important zoonotic disease affecting domestic animals and humans worldwide. The present study was undertaken on camels in and around Bikaner city of Rajasthan state of India to assess the extent of prevalence of Brucellosis in camels in this region. Since Rose Bengal Plate test (RBPT) is a serological screening method for diagnosis of Brucellosis approved by the Office International des Epizooties (OIE), RBPT was employed for detecting antibodies against *Brucella* organisms in camels. Polymerase Chain Reaction is widely followed for molecular diagnosis of several infectious diseases. DNA from whole blood of camels was analyzed by PCR for detection of *Brucella* organisms in the blood of camels. Blood samples from 177 camels (108 males and 69 females) from Bikaner and nearby villages were analyzed for Brucellosis by RBPT. Fifteen camels [7 (46.66%) males and 8 (53.33%) females] were found positive. However, none of the DNA samples from whole blood (RBPT positive or negative) from 25 camels tested was *Brucella* positive by PCR. The serological results indicate that Brucellosis is prevalent in camels and is of public health significance in Bikaner and nearby villages in Rajasthan state of India. However, detection of DNA of *Brucella* organisms in blood by PCR may not be advised for regular screening for Brucellosis since there is intermittent bacteremia in Brucellosis and *Brucella* DNA may not be detectable in blood continuously throughout the course of the disease. This reminds us that the OIE has approved RBPT, but not PCR for screening of Brucellosis.

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1. INTRODUCTION

According to the FAO [1] live animals' statistics, the worldwide camel population is about 35 million heads. As per the 20th Livestock Census of India, the total camel population in India was 2.5 Lakhs (0.25 million) in 2019. This included 0.08 million males and 0.17 million females [2]. Rajasthan had the highest camel population across India, at about 213 thousands in 2019 [3]. Camel is reared for its milk and meat. It is also used for transportation purposes.

Brucellosis is a zoonotic disease caused by bacteria of the genus *Brucella* that affects several different species of domestic animals and man. It is spread across the Mediterranean region of Europe, the Middle East, North and East Africa, Central and South Asia, and Central and South America [4]. Animals that are mostly infected by this bacterium include cattle, goats, sheep, dogs, pigs and camels among others [5]. Camels are highly susceptible to Brucellosis caused by *Brucella melitensis* and *Brucella abortus*, both of which are pathogenic to man.

Bikaner is a city in the northwest of the state of Rajasthan in India. It is located 330 kilometres northwest of the state capital, Jaipur. Bikaner city is the administrative headquarters of Bikaner district and Bikaner division. It's located on Latitude 28.027138 and Longitude 73.302155. Bikaner has a population of about 650,000 people. The present study was undertaken to determine the status of Brucellosis in camels in Bikaner district and some adjoining villages of Rajasthan state of India employing the common serodiagnostic method – Rose Bengal Plate Test (RBPT). Whole blood DNA of the camels positive by RBPT along with some RBPT negative samples were also analyzed by multiplex PCR employing Bruce Ladder.

2. MATERIALS AND METHODS

2.1 Camel Serum Samples Analyzed

A total of 177 serum samples from camels were included in this study. Out of these, 108 were males and 69 were females. The samples were collected from Bikaner and nearby villages.

2.2 Rose Bengal Plate Test (RBPT)

Equal volumes (10 µl each) of RBPT coloured antigen (Punjab Veterinary Vaccine Institute, Ludhiana) and test serum were mixed on a clean glass slide [6] with the help of a sterilized toothpick. The slide was observed for 4 min. Formation of clumps was considered a positive test while the absence of clear clumps was considered a negative reaction.

2.3 PCR Assay

Bruce Ladder PCR was employed to amplify the *Brucella* DNA from whole blood samples of camels for molecular diagnosis of Brucellosis.

0.5x TBE Working Buffer: 10x TBE: 5ml; DW: To make the volume up to 100ml.

1.5 g of agarose was dissolved in 100 ml of working buffer (0.5x TBE) and RedSafe (InfoBio) was added @10 µl per 100ml of 0.5 TBE buffer.

Lysis Buffer: 100mM KCl, 20mM TrisHCl (pH8.3), 5mM $MgCl_2$, 0.2mg of gelatin per ml and 0.9% polysorbate 20.

Genomic DNA was extracted from whole blood samples of RBPT positive or negative camels by using Genomic DNA isolation kit (Qaigen). 100 µl whole blood sample was mixed with 100 µl lysis buffer. Proteinase K was added to a final concentration of 60 µg/ml and the mixture was incubated for 60 mins at 55°C. Proteinase K was inactivated by heating the mixture to 95°C for 10 min followed by centrifugation at 12000 ×g for 10 min at 4°C. The supernatant was collected in a fresh centrifuge tube to which 0.1 volume of sodium acetate (3M) and 0.6 volume of isopropanol were added. The contents were mixed gently and kept on ice for 1 hour and then centrifuged at 8000 g for 10 min. the pellet was washed with 70% alcohol twice and dried at 37°C. Finally the pellet was suspended in 20-40 µl of Tris EDTA buffer and stored at -20°C till further use.

Bruce-ladder identification was based on the numbers and sizes of six products amplified by PCR [7]. The composition of reaction mixture was as per Table 1. Six primers were used with PCR master mix (TAKARA) in 25 µl reaction (Table 2). Each primer (forward and reverse) was used at the rate of 10 picomoles per reaction. The PCR reactions were carried out in a Thermal Cycler (BioRad) with the cycling conditions given in Table 3.

Agarose gels premixed with RedSafe examined under UV rays using the Gel Documentation System to visualize the amplified products. The amplicon size and concentrations were determined by comparing with the ladder which was run with the samples.

Table 1. Composition of the PCR reaction mixture

Component	Volume / reaction (µL)
Master Mix (Takara)	12.5
Forward Primer (10 pMoles)	0.75
Reverse Primer (10 pMoles)	0.75
DNA sample	10
Distilled water	1
Total	**25**

2.4 Analysis of PCR Products

Agarose gels were examined under UV rays using the Gel Documentation System to visualize the amplified products. The amplicon size and concentrations were determined by comparing with the molecular ladder which was run along with the samples. PCR amplified products were visualized using Agarose Gel Electrophoresis. Agarose gel (1%) was prepared using 1x Tris Acetate EDTA (TAE). Addition of Ethidium bromide (Genei^{TM}) was done to agarose at a concentration of 5µg/100ml after cooling it to 56^0C. TAE (1x) was used as casting and running buffer. 3 µl of amplified DNA was loaded using 1.5 µl of 100 bp ladder (GeneRuler 100 bp DNA Ladder-0.1 µg/µl, Thermo Scientific) was used as marker. Mini gel electrophoresis assembly (Midi submarine gel system, Genexy scientific Pvt. Ltd.), using power supply for 80 minutes was used to carry out Electrophoresis at 6.5 V/cm of gel in 1x TAE running buffer for 80 minutes. The amplified product was detected with the help of ethidium bromide in agarose gel electrophoresis. The UV transilluminator (Benchtop UV Transilluminator-BioDoc-It Imaging System, UVP) was used to visualize and automatically photograph the bands which were stored for further use.

Table 2. Oligonucleotides used in Bruce-ladder multiplex PCR assay

Primers	Sequence (5'–3')	Amplicon size (bp)	Gene	Reference
BMEI0535f	GCG CAT TCT TCG GTT ATGAA	450	bp26	Lopez-Goni et al., 2008
BMEI0536r	CGC AGG CGA AAA CAG CTA TAA			
BMEII0843f	TTT ACA CAG GCA ATC CAG CA	1071	omp31	
BMEII0844r	GCG TCC AGT TGT TGT TGA TG			
BMEI1436f	ACG CAG ACG ACC TTC GGT AT	794	Polysaccharide deacetylase	
BMEI1435r	TTT ATC CAT CGC CCT GTC AC			
BMEII0428f	GCC GCT ATT ATG TGG ACT GG	587	eryC	
BMEII0428r	AAT GAC TTC ACG GTC GTT CG			
BR0953f	GGA ACA CTA CGC CAC CTT GT	272	ABC transporter binding protein	
BR0953r	GAT GGA GCA AAC GCT GAA G			
BMEI0752f	CAG GCA AAC CCT CAG AAG C	218	rpsL	
BMEI0752r	GAT GTG GTA ACG CAC ACCAA			

Table 3. PCR cycling conditions

Stages	Steps	Temperature (ºC)	Duration	No. of cycles
I	Initial Denaturation	95	7 min	1
II	Denaturation	95	35 sec	
	Annealing	64	45 sec	25
	Extension	72	3 min	
III	Final Extension	72	6 min	1

3. RESULTS AND DISCUSSION

3.1 Analysis of Camel Sera by RBPT

Out of 177 camels, 15 were positive by RBPT (Fig. 1, Table 4). The prevalence of Brucellosis in camels was found to be 8.47% by RBPT. The positive camels included 7 (46.66%) males and 8 (53.33%) females.

In our present study on 177 serum samples from camels in and around Bikaner, prevalence by RBPT was found to be 8.47% (6.48% in males and 11.59% in females). Mean age of RBPT positive camels was 11.42 years in males and 11.12 years in females. Mathur and Bhargava [8] reported seroprevalence of Brucellosis in camels in Jorbeer village of Rajasthan to be 3.8%. Camels are usually kept mixed with ruminant species in Indian households and farms and cattle have been considered to be the potential source of infection for camels. Seroprevalence of camel brucellosis tends to follow two distinct trends with a low prevalence of below 5% in nomadic or extensively kept camels and a high prevalence of 8–15% in intensively or semi-intensively kept camels [9]. Various Biotypes trigger the infection like *B. abortus* and *B. melitensis*. Bitter [10] examined 948 camels from various herds in eastern Sudan and recorded 16.5 – 32.3 percent prevalence. Musa [11] studied 416 camels from seven herds of western Sudan owned by nomads, found a prevalence rate of 23.3 percent, and concluded that camels ranked second only to cattle in the rate of *Brucella* infection. The spread of brucellosis in camels depends on the species of *Brucella* that are prevalent in other animals in their habitat.

Khadjeh et al. [12] studied camel brucellosis in Boushehr province of Iran during the year 1997. A total of 258 serum samples were collected and serologically examined. Out of these samples, 5 cases (1.93%) showed laboratory evidence of *Brucella* infection. In bacteriological examination, the lymph nodes of all serologically positive camels were cultured. *Brucella melitensis* biotype 1 was isolated from two cultures. Dawood [13] carried out a study of the prevalence of camel brucellosis in the south province of Jordan during the years 2006 and 2007. Six hundred forty camel sera from 44 herds were randomly collected and analyzed. Rose Bengal plate test was used to screen all serum samples. The positive samples were subjected to confirmation by complement fixation test. The true prevalence of *Brucella* seropositive was 15.8%. *Brucella melitensis* biotype 3 was isolated from 2 aborted fetuses and from 2 milk samples. 64.8% of the positive camels were adults more than 4 years old and the remaining 35.2% were young ranging from 6 months to 4 years old. Abbas and Agab [9] studied the seroprevalence of brucellosis in camels. They speculated that it follows two distinct patterns: low (2-5%) prevalence in nomadic or extensively kept camels and high (8-15%) prevalence in camels kept intensively or semi-intensively.

Hadush et al. [14] conducted a cross-sectional study in three selected districts of Afar region of Ethiopia to determine the seroprevalence of camel brucellosis. A total of 1152 camels from 168 camel herds were included in the study. All serum samples were consequently tested and confirmed serologically using Rose Bengal Plate Test (RBPT) and Complement Fixation Test (CFT). Risk factors analysis was also conducted using multivariable and univariate logistic regression analysis. As a result, 58 (5.0%) were RBPT reactors in which 47 (4.1%, 95% CI: 2.9 to 5.3%) were confirmed to be positive using CFT and at least one reactor camel was found in 37 (22.0%) of the total herds sampled. The statistical analysis indicated that herd size and contact with other ruminants were the major risk factors for the presence and transmission of the disease between animals. In addition, pluriparous (4.7%), abortive (5.7%), pregnant (6.6%) and lactating (4.1%) camels were found with higher seropositivity which contributed in transmission of the disease to calves, other ruminants as well as to humans, but this was not a statistically significant association (P > 0.05).

Fig. 1. RBPT testing of serum for Brucellosis - Left: negative, Right: positive

Table 4. Camel serum samples positive for Brucellosis by RBPT

S. no.	Camel number	Age (yrs)	Sex	RBPT
1	CB1	10	Male	+
2	CB6	10	Male	+
3	CB10	8	Female	+
4	CB12	8	Male	+
5	CB16	9	Female	+
6	CB59	18	Male	+
7	CB60	18	Female	+
8	CB61	18	Female	+
9	CW14	17	Female	+
10	CW19	13	Male	+
11	CW27	13	Male	+
12	CO9	3	Female	+
13	CN3	9	Female	+
14	CN5	7	Female	+
15	CN6	8	Male	+

Our study revealed that the prevalence of Brucellosis in females was almost double that in males. It may possibly be due to the reason that the same infected male serves a large number of females in the herd. Also, natural service is widely practice instead of artificial insemination in camels. Furthermore, Brucella organisms have a predilection for placental cotyledons which are rich in erythritol required for the growth of Brucella organisms. The anatomy of the female genitalia is more conducive to retention of the microorganisms inside the long genital tract compared to males where it is largely external. Brucella organisms are secreted in semen in males and in the genital secretions and milk in case of females, besides other secretions and excretions of the body. Brucellosis causes abortion, reproductive failure, metritis, mastitis and hygroma in females and orchitis and hygroma in males.

Rose Bengal test is fast, but in its chronic form this test has many false-negative results [15]. A high proportion of animals in infected areas give results negative in RBT, but positive in CFT [16]. Different diagnostic tests have been validated for diagnosing Brucellosis in camels and humans, but only the Rose Bengal test (RBT) and the complement fixation test (CFT) are approved for diagnosis of camel and human Brucellosis in the European Union (EU) legislation on intra-community trade (Council Directive 91/68/EEC). However, there is evidence that both tests are less sensitive and specific for the diagnosis of Brucellosis in camels and humans than in cattle [16,17].

The classical Rose Bengal Plate Test (RBPT) is often used as a rapid screening test for the diagnosis of brucellosis [18,19]. The sensitivity of RBPT is high but the specificity can be disappointingly low [20]. As a result, the positive predictive value of the test is low and a positive test result thus requires confirmation by a more specific test [21]. The RBPT could sometimes give a false positive result because of S19 vaccination or of false positive serological reactions [22]. In endemic areas, there is a low level of antibody titer in normal population, making it difficult to set up a threshold to balance the sensitivity and specificity, which causes some false positive as well as false negative results. In endemic areas, there is background positivity in normal population, which may lead to false positive results. The positivity of agglutination test decreases as the disease prolongs. Even in acute stage with positive bacterial culture, patients can have false negative results. Another potential problem for agglutination test is the presence of cross reactivity with other bacteria, such as *Yersinia enterocolitica, Salmonella urbana* group N, *Vibrio cholera, Escherichia coli* O:157 and *Francisella tularensis*, causing false positivity. Gram negative bacteria such as *Campylobacter fetus* and *Bordetella bronchiseptica* may cross react with smooth *Brucella* spp. [23].

3.2 PCR on Camel DNA

PCR using DNA from *B. abortus* strains (positive controls) amplified five fragments of 1682, 794, 587, 450, and 152 bp in size; with *B. melitensis* DNA, an additional 1071 bp fragment was amplified. However, none of the 25 samples from RBPT positive or negative camels was positive for *Brucella* (Table 5, Fig. 2). RBPT, ELISA and

PCR are the most widely used tests for the laboratory diagnosis of Brucellosis in cattle, camel and humans [24]. Alshaikh et al. [25] employed Polymerase chain reaction (PCR) to diagnose brucellosis in camels. DNA was extracted from 26 serum samples of camels that tested positive for brucellosis by two or more serologic tests. PCR products indicating the presence of a *Brucella* spp. DNA were detected in all samples. In our present study, none of the whole blood DNA samples of 25 camels tested were positive by PCR. A possible reason of seropositivity of cases negative by PCR in our present study could be elimination of the pathogen from the body by the immune system. The antibody is known to persist in blood long after the removal of the pathogen from the body. Alternatively, the initial bacterial load after infection may be just enough to induce antibody response by antigenic stimulation but not adequate enough to cause a full blown disease along with detectable bacteremia. Thus, the quantum of bacterial load and the magnitude of individual immune response may be the factors responsible for incoherence between the results of serological and molecular assays.

Chothe et al. [26] carried out a study on 200 samples from cattle suspected of Brucellosis to ascertain the effectiveness and suitability of PCR and commercially available ELISA kits. In PCR, only 3 out the 200 sample showed a band of size 905bp, typical of *Brucella* species. Other samples failed to show positive reaction by PCR. In comparison, ELISA could detect 75 out of the 200 samples as positive. They concluded that ELISA is a better confirmatory test than PCR for screening animals for Brucellosis. In Brucellosis, bacteremia is not persistent throughout the course of the disease. There are waves of bacteremia alternated by phases of latency when the microorganisms hide inside the phagocytes in the reticuloendothelial system. This is manifested in the form of intermittent fever. If blood is drawn from the infected individual during the latent phase, it may not contain bacteria and hence no microbial DNA can be detected in this sample by molecular techniques even if the microorganisms are present in the body. On the other hand, a single antigenic exposure of the immune system may induce detectable antibodies against the pathogen for a considerable period of time.

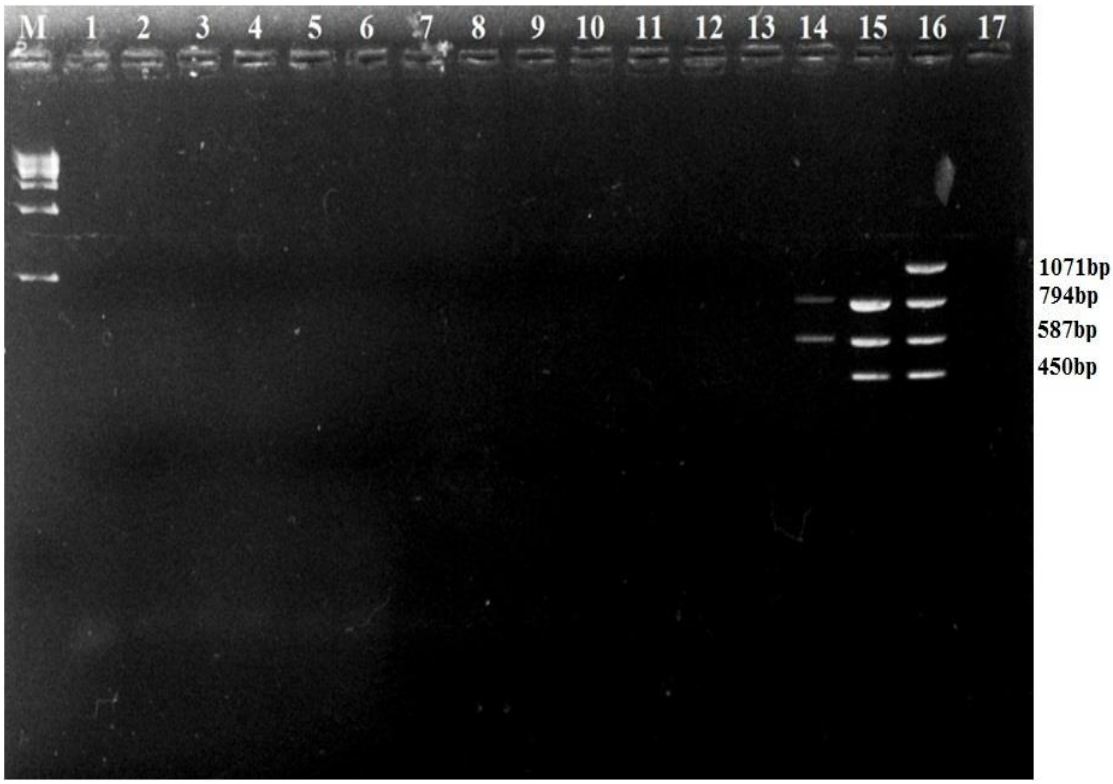

Fig. 2a. Bruce ladder PCR of camel whole blood DNA samples. Lane M = marker; Lanes 14, 15 & 16 = positive controls; Lanes 1-13 = camel samples 1-13

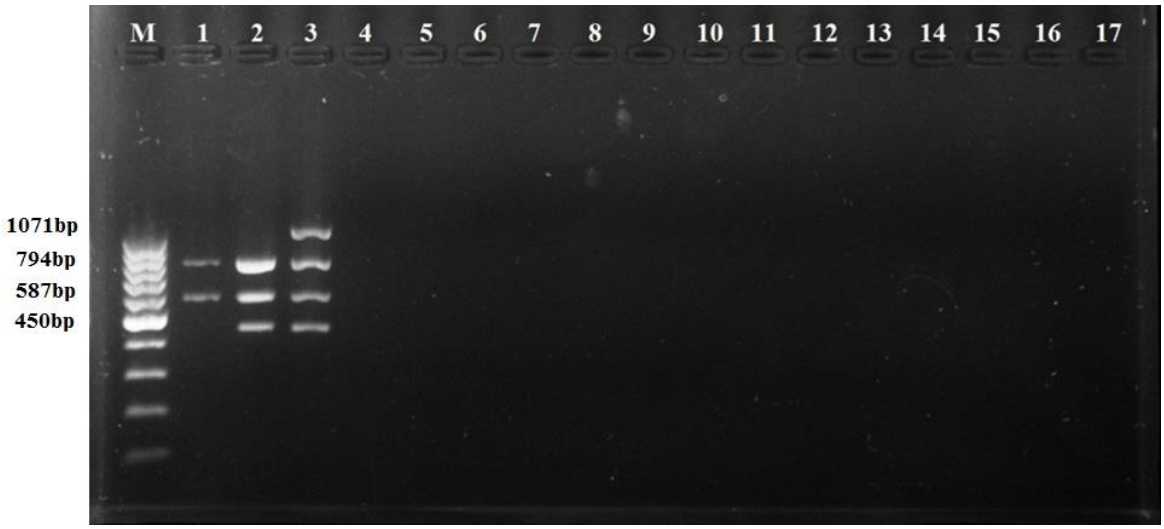

Fig. 2b. Bruce ladder PCR of camel whole blood DNA samples. Lane M = marker; Lanes 1, 2 & 3 = positive controls; Lanes 4 -17 = camel samples 14 to 25

Table 5. Camel blood samples analyzed by PCR

S. no.	Sample no.	RBPT	PCR	Age (years)	Location	Sex
1	CB15	Negative	Negative	5	Bikaner	Female
2	CB2	Negative	Negative	10	Bikaner	Male
3	CB34	Negative	Negative	12	Bikaner	Male
4	CB4	Negative	Negative	10	Bikaner	Male
5	CB5	Negative	Negative	10	Bikaner	Male
6	CB7	Negative	Negative	10	Bikaner	Male
7	CB8	Negative	Negative	0.3	Bikaner	Female
8	CB9	Negative	Negative	9	Bikaner	Female
9	CB11	Negative	Negative	8	Bikaner	Male
10	CB13	Negative	Negative	8	Bikaner	Male
11	CB16	Positive	Negative	9	Bikaner	Female
12	CB1	Positive	Negative	10	Bikaner	Male
13	CB6	Positive	Negative	10	Bikaner	Male
14	CB59	Positive	Negative	18	Bikaner	Male
15	CB61	Positive	Negative	18	Bikaner	Female
16	CB10	Positive	Negative	8	Bikaner	Female
17	CB60	Positive	Negative	18	Bikaner	Female
18	CB12	Positive	Negative	8	Bikaner	Male
19	CW14	Positive	Negative	17	Gadwala	Female
20	CW19	Positive	Negative	13	Gadwala	Male
21	CW27	Positive	Negative	13	Gadwala	Male
22	CO9	Positive	Negative	3	Gadola	Female
23	CN3	Positive	Negative	9	Naurangdesar	Female
24	CN5	Positive	Negative	7	Naurangdesar	Female
25	CN6	Positive	Negative	8	Naurangdesar	Male

PCR is a very sensitive technique [27]. An evaluation of Bruce-ladder multiplex PCR assay was performed in seven laboratories using 625 *Brucella* strains from different animal and geographical origins. This test could differentiate in a single step all of the classical Brucella species, including the S19, RB51, and Rev.1 vaccine strains. A major advantage of the Bruce-ladder PCR assay over other multiplex PCR tests is that it can identify and differentiate all of the *Brucella* species and the vaccine strains in the same test. Bruce-ladder PCR can be a useful tool for the rapid identification of *Brucella* strains of animal or human origin [7].

A rapid, simple and accurate diagnostic test for Brucellosis would lead to rapid, early and easy diagnosis and effective control of the disease, preventing its spread in the community and averting the huge losses to farm economy. It is advisable that instead of relying on a single cumbersome and expensive diagnostic test, a combination of two or more simple, cheaper and sensitive tests, like RBPT and ELISA may be adopted for more accurate and rapid results. This has already been recommended in case of bovine Brucellosis by Chachra et al [28] and Chothe et al [26].

4. CONCLUSIONS

Brucellosis is prevalent in camels in Bikaner and nearby villages in Rajasthan state of India as revealed by serological screening with RBPT. However, detection of DNA of *Brucella* organisms in blood by PCR may not be advised for regular screening for Brucellosis since there is intermittent bacteremia in Brucellosis and *Brucella* DNA may not be detectable in blood continuously throughout the course of the disease.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO. Dairy Production and Products: Camels; 2019. Available:www.fao.org/dairy-production-products/production/dairy-animals/camels/en/
2. Vikaspedia; 2021. Available:https://vikaspedia.in/agriculture/agri-directory/reports-and-policy-briefs/20th-livestock-census
3. Statista; 2021. Available:https://www.statista.com/statistics/1078163/camel-inventory-by-state-india/
4. Corbel M. Brucellosis in humans and animals. WHO Press, World Health organisation, Geneva, Switzerland; 2006.
5. CDC. Brucellosis. Centers for Disease Control and Prevention, Atlanta; 2019. Available:www.cdc.gov/brucellosis/index.html
6. Morgan WJB, Mackinnon DT, Gill KPW, Gower SGM, Norris PIW. Brucellosis diagnosis: Standard Laboratory Techniques Report Series no. 1, Weybridge, England; 1978.
7. Lopez-Goni I, García-Yoldi D, Marín CM, de Miguel MJ, Mun˜oz PM, Blasco JM, Jacques I, Grayon M, Cloeckaert A, Ferreira AC, Cardoso R, Corre^a de Sa` MI, Walravens K, Albert D, Garin-Bastuji B. Evaluation of a Multiplex PCR Assay (Bruce-ladder) for molecular typing of all *Brucella* species, including the vaccine strains. Journal of Clinical Microbiology. 2008;46(10):3484–3487.
8. Mathur KN, Bhargava SC. Sero-prevalence of Q fever and brucellosis in camels of Jorbeer and Bikaner, Rajasthan State. Indian Journal of Medical Research. 1979;70(11):391–393.
9. Abbas B, Agab H. A review of camel brucellosis. Preventive Veterinary Medicine. 2002;55:47–56.
10. Bitter H. Disease resistance in dromedaries with particular reference to *Trypanosoma evansi* infection. Inaugural Desertation. Tierarticliche Hochschedule, Hanover, Germany.; Cited in: Abbas B, Agab H. A Review of Camel Brucellosis. Preventive Veterinary Medicine. 1986;55(1):47-56.
11. Musa MT. Brucellosis in darfur states: The magnitude of the problem and methods of diagnosis and control of the disease. PhD Thesis, University of Khartoum, Sudan. 1995;83-96.
12. Khadjeh G, Zowghi E, Zarif-Fard M. Incidence of brucellosis in one-humped camels of Boushehr, Iran. Archives of Razi Institute. 1999;50:83–86.
13. Dawood HA. Brucellosis in camels (*Camelus dromedorius*) in the South Province of Jordan. Journal of Agricultural and Biological Sciences. 2008;3(3):623-626.
14. Hadush A, Pal M, Kassa T, Zeru F. Sero-epidemiology of camel brucellosis in the Afar region of Northeast Ethiopia. Academic Journal. 2013;5:269–275. Available:https://doi.org/10.5897/JVMAH13.0235
15. Roushan MR, Amin MJ, Abdoel TH, Smits HL. Application of a user-friendly Brucella-specific IgM and IgG antibody assay for the rapid confirmation of Rose Bengal-positive patients in a hospital in Iran. Transactions of the Royal Society for Tropical Medicine and Hygiene. 2005; 99:744–750.

16. Blasco JM, Marin C, Jimenez de Bagues M, Barberan M, Hernandez A, Molina L, Velasco J, Diaz R, Moriyon I. Evaluation of allergic and serological tests for diagnosing *Brucella melitensis* infection in sheep. Journal of Clinical Microbiology. 1994;32:1835–1840.
17. Garin-Bastuji B, Blasco JM, Grayon M, Verger JM. *Brucella melitensis* infection in sheep: present and future. Veterinary Research. 1998;29(3/4):255-274.
18. Oomen LJ, Waghela S. The rose bengal plate test in human brucellosis. Trop. Geog. Med. 1974;26(3):300-302.
19. Ruiz-Mesa JD, Gonzalez JS, Reguera JM, Martin L, Palmero SL, Colmenero JD. Rose bengal test: Diagnostic yield and use for the rapid diagnosis of human brucellosis in emergency departments in endemic areas. Clin. Microbiol. Inf. 2005;11(3):221-225.
20. Barroso GP, Pelayo RC, Extremera G. Study of 1595 brucellosis cases in the Almeria province (1972–1998) based on epidemiological data from disease reporting. Review de Clinics de Espana. 2002;202:577–582.
21. Smits HL, Kadri SM. Brucellosis in India: A deceptive infectious disease. Indian Journal of Medical Research. 2005; 122:375-384.
22. OIE. Manual of diagnostic tests and vaccines for terrestrial animals. World Organisation for Animal Health, Paris, France; 2009.
23. Corbel MJ. Recent advances in the study of *Brucella* antigens and their serological cross reactions. Vet. Bull. 1985;55:927-942.
24. Alton GG. *Brucella melitensis*. In: Nielsen K and Duncan J R. (eds.) Animal Brucellosis. CRC Press Inc., Boca Raton, Florida. 1990;383–409.
25. Alshaikh MA, Aljumaah R, Alhaidary AA, Korashi MA. Camel brucellosis in Riyadh region, Saudi Arabia. Journal of Camel Practice and Research. 2007;14(2):113-117.
26. Chothe S, Saxena HM, Chachra D. Comparative evaluation of PCR and ELISA for diagnosing bovine Brucellosis. Intas Polivet. 2013;14(1):80-84.
27. Amin AS, Hamdy ME, Ibrahim AK. Detection of *Brucella melitensis* in semen using polymerase chain reaction assay. Veterinary Microbiology. 2001;83:37-44.
28. Chachra D, Saxena HM, Kaur G, Chandra M. Comparative efficacy of rose bengal plate test, standard tube aggulutination test and dot-ELISA in immunological detection of antibodies to *Brucella abortus* in sera. Journal of Bacteriology Research. 2009;1(3):30-33.

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