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Serological Survey of Brucellosis in Camel of Gujarat

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A B S T R A C T

Brucellosis is a zoonotic bacterial disease of ruminants and also reported from camel. Camels are one of the most important sources of livelihood for the nomadic population of Gujarat. There are limited published information regarding the sero-epidemiology of camel brucellosis in Gujarat. Therefore, present study was aim to determine the brucella specific antibodies in camel from three camel rearing districts of Gujarat using RBPT, i-ELISA and MRT. On screening of 352 serum samples, 41(11.64%) and 16 (4.54%) samples found positive by RBPT and i-ELISA, respectively. 8 (15.38%) samples of milk found positive from total 52 samples by MRT. It was also revealed from the study that the female camel have high seroprevalence of brucellosis than male. Prevalence of brucellosis among the camels of various districts of north Gujarat indicates that the disease is endemic and may act as the source of infection for other susceptible animals including human beings.

Introduction

Brucellosis is caused by various species of the genus *Brucella*, which is the second most widely spread zoonosis worldwide (Dawood, 2008). It is one of the infectious diseases, which poses major constraint for animal production. The disease is an important public health problem in many parts of the world including India (Pal, 2007; Hadush and Pal, 2013). Brucellosis is burning problem in Gujarat, where cases of human brucellosis are reported along with high sero-prevalence in animals.

Brucella can affect almost all domestic species and cross transmission can occur between cattle, sheep, goat, camel and other species (Ghanem *et al.*, 2009). Brucellosis was reported in camels as early as 1931 (Solonitsuin, 1949); since then, the disease has been reported from all camel-keeping countries (Gwida *et al.*, 2012). Camels are frequently infected with *Brucella* organisms, especially when they are in contact with infected large and small ruminants (FAO/WHO, 1986; Radwan *et al.*, 1992). Brucellosis is characterized by abortion and birth of non-visible offspring in females; orchitis and epididymitis in males (Radostits *et al.*, 2007). The disease is also associated with infertility and prolonged calving intervals and has considerable impact on camel production. It may also causes, chronic inflammation of joints, tendon sheath and synovial bursa especially at the carpus (Abbas

and Agab, 2002). Serological evidence for *Brucella* infection in camels has been reported from Asia and Africa (Dawood, 2008). There are many factors that can affect the prevalence of brucellosis in various species of livestock. Prevalence may vary according to climatic conditions, geography, species, sex, age and diagnostic tests used (Gul and Khan, 2007). Camels are not known to be primary host for any of *Brucella* organisms but they are susceptible to both *B. abortus* and *B. melitensis* (Musa and Shigidi, 2001). The relation between *Brucella* infection and abortion in camels has been recorded (Al-Majali *et al.*, 2008; Higgins, 1986, Agab and Abbas, 1999). Both *Brucella abortus* and *Brucella melitenis* have been isolated from fetuses, genital discharges, urine and milk (FAO/WHO, 1986).

The disease has been reported from various species in different parts of India. But, there are limited published reports of brucellosis in camel and only a few reports from Gujarat. Camel brucellosis has not received proper attention from researchers and scientists. Therefore, present study was planned for the detection of *Brucella* antibody from camels and overall seroprevalence of camel brucellosis was determined using RBPT, iELISA and MRT.

Materials and Methods

The present study was conducted during the period from June to December 2014. A total of 352 serum samples were collected from camel of rural areas and organized farms belongs to three semi-arid districts (Banaskantha, Patan and Kutch) of north Gujarat, having high camel population. Blood samples were collected in tube containing clot activator (Greiner bio-one, Austria) aseptically from the jugular vein and allowed to clot. Serum was collected and stored at -20ºC till further use.

All the collected serum samples were screened for antibodies against *Brucella* by the Rose Bengal Plate Test (RBPT) and the Indirect Enzyme Linked Immunosorbant Assay (i-ELISA). The RBPT antigen was procured from the Institute of Animal Health and Veterinary Biologicals (IAH and VB), Hebbal, Bangalore, Karnataka. RBPT was performed according to manufacturer's instructions. Definite clumping/agglutination was considered as positive reaction; whereas, no clumping/agglutination was considered as negative. Indirect ELISA kit was procured from National institute of Veterinary Epidemiology and Disease informatics (NIVEDI) Bangalore, and used as per manufacturer's protocols. The kit detects the antibodies against *Brucella* lipopolysaccharide (LPS) in serum samples of *B. aborus* and *B. melitensis.*

Milk ring test (MRT)/Aborts Bang ring test (ABR) was performed using colored antigen procured from Institute of Animal Health and Veterinary Biologicals (IAH and VB), Hebbal, Bangalore, Karnataka. In brief, MRT antigen was brought to room temperature before use. 2 ml of milk sample was taken in test tube. Then, 2 drops of MRT/ABR antigen was added, and gently mixed. The tubes were incubated at 37^{o}C for one hour. Then kept at room temperature for 30 min. and results were recorded. A sample which shows colored ring at the surface was considered as positive (Fig. 2).

The genomic DNA from 22 serum samples was extracted using DNeasy blood and tissue kit (QiAgen, USA) and subjected for *Brucella* detection using primer pair (Table 1).

PCR reaction mixture was prepared in 25µl volume consisting of 12.5 µl 2x PCR-Master-Mix (Fermentas), 1 µl of forward and reverse primers each (12pmol/µl), 10µl of DNA template and nuclease free water. PCR

amplified product was checked by running samples over 1.5% agarose gel.

Results and Discussion

According to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization for Animal Health (OIE) brucellosis is still one of the most important and widespread zoonoses in the world (Young, 1995). It has high public health significance and may poses threat to all human as diseases may transmit through consumption of raw and under cooked milk and milk products (Schelling *et al.,* 2003).

The present study was aimed to study the prevalence of brucellosis in camel. Total 352 serum samples were screened, 41(11.64%) were found positive by RBPT (Table 2). In accordance to the present finding, Zewold and Haileselassie (2012) reported 11.90 percent seroprevelence by RBPT (Fig. 1). However, in-contrast to present findings, lower rate of seroprevalence was reported (Ghanem *et al.*, 2009; Shome *et al.*, 2013); whereas, Dawood (2008) reported higher rate (14.2 %) of seroprevelence of *Brucella* antibody in camel by RBPT. In indirect ELISA, 16 (04.54%) were found positive (Table 2). In accordance to the present findings, Shome *et al.*, (2013) reported 4.9 percent seroprevelence using i-ELISA. However, in contrast to present findings, Ghanem *et al.*, (2009) reported lower rate of 3.1 percent seroprevalence. The difference in seroprevalence might be due to differences in sample size, tests used, management condition, herd size or due difference in seroprevalence in the two study areas. According to Radiostits *et al.*, (2007), herd size and management condition determine rate of transmission of *Brucella* infection in different study areas. In district wise analysis of results, it was found that the Banakantha, Patan and Kutch have 16.89, 11.57, 02.41 and 5.40, 05.78, 01.20 % seroprevalence using RBPT and i-ELISA, respectively (Table 2 and Fig. 3).

In male camels, seroprevalence reported as 09.45 and 01.35 percent by RBPT and i-ELISA, respectively. Whereas, in case of female, it was 12.23 and 05.38 per cent by RBPT and i-ELISA, respectively (Table 2). The higher seroprevalence of brucellosis in female camels might be attributed to the fact that female animals remained in the breeding herds for a longer period of time than male animals that are being culled from time to time and sold to meet pastoralists' financial needs. According to Radostitis *et al.*, (2007), physiological and behavioral differences between male and female animals involve in the variation in sex susceptibly for brucellosis. *Brucella* infection is more common in female camels may be associated to erythritol (Gyles *et al.*, 2004).

Table.1 Primer pair for *Brucella* detection

Sr. No	Primer	Forward /Reverse	Sequence (5'-3')	Product size (bp)	Reference
1.	B4	Forward	TGG CTC GGT TGC CAA TAT CAA	223	Bailly *et al.* (1992)
2.	B5	Reverse	CGC GCT TGC CTT TCA GGT CTG		

Table.2 Seroprevalence of *Brucella* in camel by RBPT and i-ELISA

Attributes:	Sample tested	No. of sample found positive			
		RBPT	%	i-ELISA	%
Districtswise Seroprevalence:					
Banaskantha	148	25	16.89	08	05.40
Patan	121	14	11.57	07	05.78
Kutch	83	02	02.41	01	01.20
Total	**352**	**41**	**11.64**	**16**	**04.54**
Sexwise Seroprevalence:					
Male	74	07	09.45	01	01.35
Female	278	34	12.23	15	05.38
Total	**352**	**41**	**11.64**	**16**	**04.54**

Table.3 Seroprevalence of *Brucella* in camel by MRT

Districts	Sample tested	MRT (%) positive
Banaskantha	22	05 (22.73)
Patan	19	03 (15.79)
Kutch	11	00 (0.0)
Total	**52**	**08 (15.38)**

Fig.1 Rose Bengal Plate Test (RBPT)

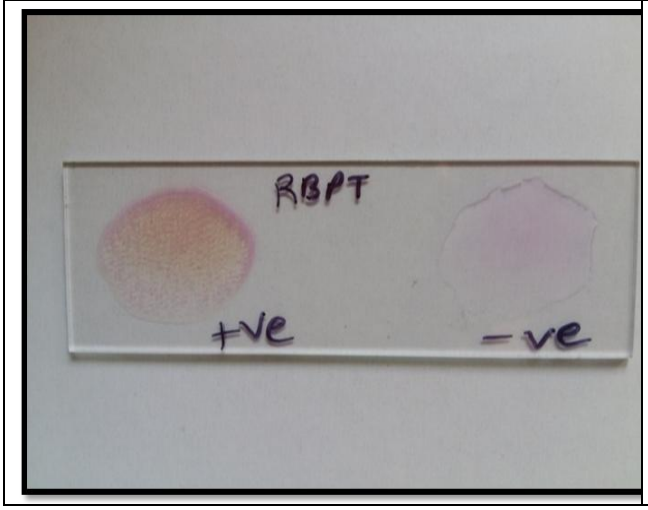


Fig.2 Milk ring test for the detection of *Brucella* antibodies

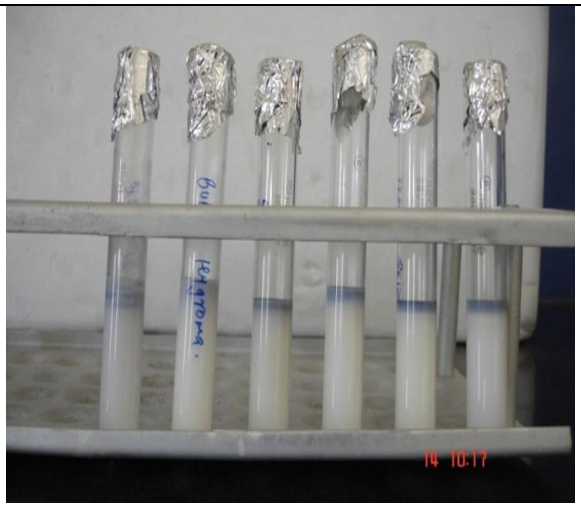
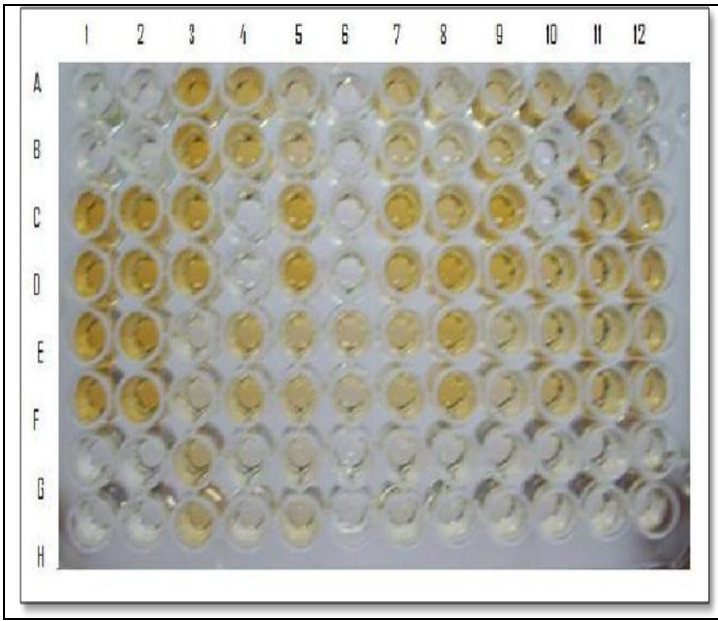


Fig.3 Microtiter ELISA plate showing result of i-ELISA



Wells A1, B1, A2, B2: Negative control

Wells C1, D1, C2, D2: Moderately positive control

Wells E1, F1, E2, F2 : Strong positive control

Wells A3, B3, C3, D3, C5, D5 etc. field sera indicate positive reaction

A total of fifty two (52) camel milk samples were tested using the Milk Ring Test (MRT) and eight (08) samples (15.38%) were found positive for the presence of *Brucella* specific antibodies (Table 3). Similar observation was also reported by Wanjohi *et al.,* (2012). High prevalence in the present study might be due to the collection of samples from the endemic population and have history of abortion. Moreover, rearing of multispecies in same herd may leads to close contact of animals, which may facilitate the exchange of various pathogenic microorganisms. In the present study area, it was observed that the camels were reared with small ruminants like sheep and goats which might be the possible source of infection for camel. Which may be the possible source of abortion in camels and similar observations were reported from different places (Gameel *et al.,* 1993; Radwan *et al.,* 1995).

Several studies have documented the detection of *Brucella* specific genome from the serum samples using PCR (Shome *et al.,* 2013; Brown *et al.,* 1995; Murdoch *et al.,* 1996; Bougnoux *et al.,* 1999; Kawamura *et al.,* 1999). The use of serum instead of whole-blood samples offers several advantages for nucleic acid amplification. In the present study, an attempt was made to detect the *Brucella* specific antigen from the serum samples using PCR. But it not observed that the specific amplification in any samples. This might be possible due to small sample size and time of sample collection.

The present study may be concluded that brucellosis is prevalent in major camel rearing districts of Gujarat. Therefore, individuals associated with camel rearing and managements need to be aware regarding the possible source of *Brucella* infection. Therefore, it's worth to include this species in state/national *Brucella* control programme, which may help to improve the production and minimize risk of transmission to humans.

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