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# A REPORT OF SEROPREVALENCE OF CAMEL BRUCELLOSIS IN INDIA

Rajeswari Shome<sup>1</sup>, V.K. Gupta<sup>2</sup>, B. Bhardwaj<sup>3</sup>, B.R. Shome<sup>4</sup>, M. Nagalingam<sup>5</sup> and H. Rahman<sup>6</sup>

<sup>1,5,6</sup>Project Directorate on Animal Disease Monitoring and Surveillance (PD\_ADMAS), Hebbal, Bangalore 560 024, India;

<sup>2</sup>Central Institute for Research on Goats, Makhdoom, Mathura, Uttar Pradesh 281 122, India,

<sup>3</sup>Department of Animal Husbandry, Government of Rajasthan, Udaipur, Rajasthan, India

## ABSTRACT

Among 78 camel serum samples tested, 7(8.9%) and 3 each (4.9%) were positive for anti Brucella antibodies by RBPT, ELISA and SAT, respectively. Interestingly, in PCR, one female camel out of 42 camel sera DNA amplified 223bp product specific to *Brucella* genus.

**Key words:** Brucellosis, camel, Indian report, sero-surveillance

Brucellosis is a serious zoonotic disease affecting man and all domestic animals including camel. Camel brucellosis has been reported as early as 1931 (Solonitsuin, 1949); since then, the disease has been reported from all camel-keeping countries (Gwida *et al*, 2012). Camels are not known to be primary hosts of Brucella, but they are susceptible to both *B. abortus* and *B. melitensis* (Cooper, 1991), especially when they are in contact with infected large and small ruminants (Radwan *et al*, 1992). Thus, the infection of camel herds depends on the *Brucella* species prevalent in other animal species sharing the same habitats and on husbandry methods (Musa *et al*, 2008). The conditions caused by the disease were retention of placenta, foetal death, mummification, delayed maturity and infertility. Often typical clinical signs of brucellosis in camels are lacking and diagnostic methods are not fully evaluated yet. The present study reports brucellosis in camels by different serological tests and PCR.

## Materials and Methods

Seventy eight serum samples (females, n=73 and males, n=5) collected from camel herds with a history of abortion were received from Rajasthan state of India for investigation of brucellosis during 2008-2012. All camels were sexually matured and single humped, reared in semi-intensive system.

The serum samples were initially subjected to rose bengal plate test (RBPT) and serum agglutination test (SAT) as per Alton *et al* (1975). The *B. abortus* coloured and plain antigens for RBPT and SAT were procured from the Institute of Animal health and

Veterinary Biological (IAH & VB), Bangalore, India. Serum samples exhibiting any degree of clumping of coloured antigen in RBPT and titre of >1:40 (80 IU/ml) was considered positive for Brucella antibodies in SAT (Al Dahouk *et al*, 2003).

Indirect ELISA to detect anti-brucella antibodies was carried out using smooth lipopolysaccharide (sLPS) antigen as per the iELISA protocol described in OIE manual (OIE, 2009) using recombinant protein-G conjugate based indirect ELISA standardised and being regularly used in our laboratory (Shome *et al*, 2011). Briefly, 1: 300 dilution sLPS (10ng/well) was prepared in carbonate- bicarbonate coating buffer (pH-9.6). To which 100 µl of the diluted antigen was added to each well and incubated at 37°C for 1hr. The contents were then emptied and washed three times in wash buffer (PBS containing 0.05% Tween20) and dried by tapping on clean towel. One hundred microlitre of 1:100 dilutions of test and bovine control sera were prepared in blocking buffer and then transferred to respective wells of the coated plate in duplicate for test sera and quadruplicate for control sera and incubated at 37°C for 1 hr on plate shaker (200rpm/minute). Washing and drying of plate was done as described earlier. One hundred µl of 1:8000 dilution of recombinant protein-G HRP conjugate which can detect all major livestock species immunoglobulins (Pierce) in blocking-cum-dilution buffer was added and incubated for an hour as described earlier. Washing and drying was repeated as described. One hundred µl of chromogen-substrate solution (containing 5mg OPD tablet in 12.5 ml distilled water and 50µl of 3% H<sub>2</sub>O<sub>2</sub>) was added and

SEND REPRINT REQUEST TO V. K. GUPTA email: vivekgupta@scientist.com

incubated for 10 minutes at room temperature. Fifty  $\mu$ l of 1M  $H_2SO_4$  was added to stop enzyme- substrate reaction. The absorbance values were read at 492 nm using an ELISA micro plate reader and the per cent positivity (pp) values are calculated as follows

$$PP = \frac{\text{Average OD values of test sera} \times 100}{\text{Median OD value of strong positive control sera}}$$

The cutoff PP value of 55 and above was considered seropositive for brucellosis.

The genomic DNA from forty two serum samples was extracted using DNeasy blood and tissue kit protocol (QiAgen, USA). The following primer pairs were used for the identification of genus *Brucella*: B4/B5 {(B4(F) TGGCTCGGTTGCCAATATCAA B5(R) CGCGC TTGCCTTTCAGGTCTG} for the amplification of 223 bp product as per Baily *et al* (1992). PCR reaction mixture was prepared in 25 $\mu$ l volume consisting of 12.5  $\mu$ l 2x PCR-Master-Mix (Fermentas), 1  $\mu$ l of forward and reverse primers (12pmol/ $\mu$ l), 10 $\mu$ l of DNA template and nuclease free water and PCR product was analysed by 1.5% agarose gel electrophoresis stained with ethidium bromide.

## Results and Discussion

There are few reports covering the prevalence of camel brucellosis in India which has stimulated us to work in this area (Mathur and Bhargava, 1979). Among 78 camel serum samples tested, 7(8.9%) and 3 each (4.9%) were positive for anti *Brucella* antibodies by RBPT, ELISA and SAT, respectively (Table 1). Interestingly, in PCR, one female camel out of 42 serum DNA tested, amplified 223bp product specific to *Brucella* genus (Fig 1). The PCR positive sample from camel had history of abortion fortnight back and detection of both *Brucella* antigen by PCR in serum (in the absence of clinical samples for isolation) along with seropositivity confirmed abortions in camel herd due to brucellosis. Though, seven samples were ELISA positive, only one serum sample was PCR positive. This might be due to the intracellular nature of *Brucella* which normally reside in various joints and internal organs or due to the absence of bacteria in serum during chronic infection (Morata *et*

*al*,1998). There are reports of *Brucella* DNA detection in serum samples by PCR (Gupta *et al*, 2009; Shome *et al*, 2011; Vivekananda *et al*, 2012) and it has been stated that serum is preferred sample for the detection of *Brucella* DNA (Zerva *et al*, 2001). One male camel was seropositive among the samples tested and this may play great role in disease transmission within the herds because of using the common male animal for natural breeding.

None of these previous studies examined the possibility of amplifying *Brucella* DNA in camel serum samples. However, the use of serum instead of whole-blood samples offers several advantages for nucleic acid amplification methods. Inhibition by anticoagulants, hemoglobin, or any other substance present in whole blood but not in serum is circumvented. Red blood cell lysis, washings by centrifugation and measurement and adjustment of isolated DNA concentrations are not required. Overall, the procedure is simplified and turn around time is shorter, while sensitivity may be increased. Regarding the origin of pathogen nucleic acids in serum samples, most probably they are released in the circulation as breakdown products during bacteraemia. Several studies have documented the presence of circulating pathogen DNA in serum samples (Brown *et al*, 1995; Murdoch *et al*, 1996; Bougnoux *et al*, 1999; Kawamura *et al*, 1999).

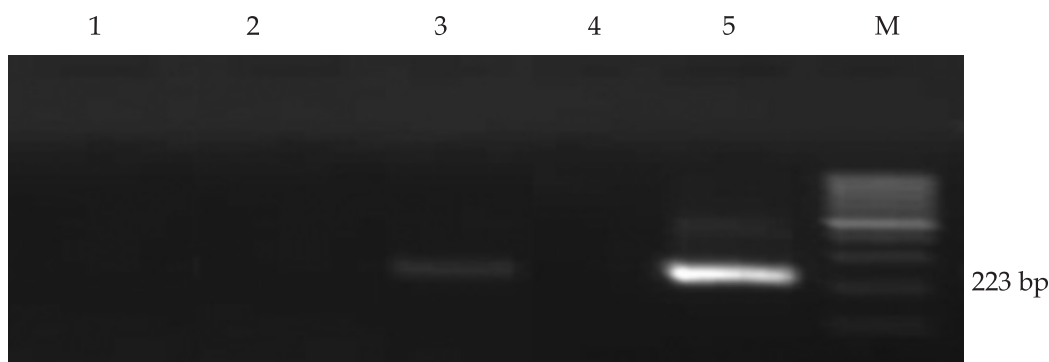
Serological evidence of 3.8% to 5.2% for *Brucella* infection in one-humped camels has been reported from India (Mathur and Bhargava, 1979) in the Rajasthan region of India. In the present study, though one lot of samples were not tested for SAT, ELISA and PCR, the overall seroprevalence was found 8.9% and this high prevalence may due to the reason that they were collected from camel herds suspected for brucellosis. The serological surveys have shown different prevalence rates from different countries i.e. Egypt 10 to 20% (Hamada *et al*, 1963), Ethiopia 51% (Richard, 1975), Chad 33% (Blaizot, 1976), Nigeria 1.3% (Okoh, 1979), Somalia 10.4% (Andriani *et al*, 1983) and Sudan 4.9% (Abu Damir *et al*, 1984).

The camels are always herded together with sheep and goats and to a lesser extent with cattle and

**Table 1.** Diagnosis of camel brucellosis by different diagnostic tests.

Sample batches	No of camel serum samples	Sex	RBPT positive	SAT titres > 1;40	ELISA positive	PCR
Lot No.1	27	F	2	2	2	-ve
Lot No.2	15	F	1	1	1	+ve*
Lot No.3	36	M -5 F- 31	4 ( M -1 F- 3)	ND	ND	ND
Total	78		7	3	3	1

\*history of abortion two weeks back; ND –not done



**Fig 1.** *Brucella* genus specific PCR: Lane 1&2, negative serum samples; Lane 3, positive serum sample; Lane 4, known negative control; Lane 5, known positive control; Lane M, 100bp DNA ladder.

they share the same watering points and pastures, and so it is not surprising to find a higher incidence of the disease among camels (Teshome *et al*, 2003). Seroprevalence of camel brucellosis appear to follow two distinct patterns, a low prevalence below 5% in nomadic or extensively kept camels and high prevalence 8–15% in camel kept intensively or semi intensively (Abbas and Agab, 2002).

The disease prevalence also depends on husbandry and management practices, presence of reactor animals in the region, continuous movement of infected camels, absence of veterinary service and lack of awareness about the disease in camels. High animal and herd prevalence have been reported from numerous countries, which not only pose a continuous risk for human infection, but also increase the spread of infection through uncontrolled trade of clinically suspected animals. Abortion has been reported in camels due to *B. melitensis* and the bacteria have been isolated from aborted fetuses, genital discharge, urine and milk (Gameel *et al*, 1993; Radwan *et al*, 1995). Intervention strategies should include safe breeding procedures, regular serology testing, slaughtering of infected animals, vaccination of uninfected herds of camels using *B. abortus* strain S19 and *B. melitensis* strain Rev.1 and improving management practices and movement control (Wernery and Kaaden, 2002).

The camel milk is consumed raw by camel keeper and positive effect of camel milk on diabetic patients has been studied in India (Agrawal *et al*, 2005). This clearly indicates that camel brucellosis is as hazardous as that of bovine and small ruminants. Therefore, pasteurisation or proper boiling of milk is adequate to prevent transmissions of diseases especially brucellosis through milk. However, infected male and female camels act as disease transmitter, public health nuisance and need to be identified to eliminate the spread of the disease.

Further studies comprising of structured sample screening in camel herds and isolation will definitely help to map the disease prevalence in camel rearing states. Of course sensitisation to avoid consumption of raw milk will minimise disease incidence in humans. The presence of *Brucella* DNA as demonstrated by PCR or presence of anti *Brucella* antibodies proved by different serological tests proved a potential risk of camel owner and consumers who are consuming products of these animals.

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