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A REPORT OF SEROPREVALENCE OF CAMEL BRUCELLOSIS IN INDIA

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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 antibrucella 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_2O_2) was added and

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incubated for 10 minutes at room temperature. Fifty µl 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

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

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 25µl volume consisting of 12.5 µl 2x PCR-Master-Mix (Fermentas), 1 µl of forward and reverse primers (12pmol/µl), 10µl 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

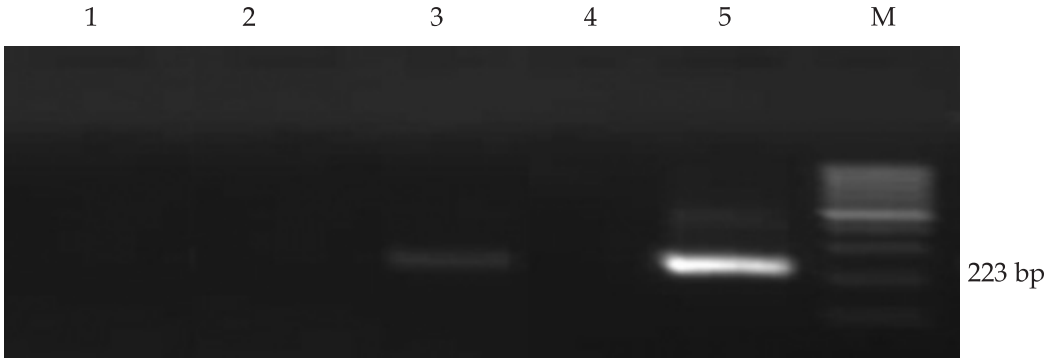

Fig 1. Brucella genus specific PCR: Lane 1&2, negative serum samples; Lane 3, positive serum sample; Lane 4, known negative control; Lane 5, known positive control; Lane M, 100bp DNA ladder.

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 foetuses, 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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References

Abbas B and Agab H (2002). A review of camel brucellosis. Preventive Veterinary Medicine 55:47-56.

Abu Damir H, Kenyon SJ, Khalaf Alla AE and Idris OF (1984). Brucella antibodies in Sudanese camels. Tropical Animal Health and Production 16:209-212.

Agarwal RP, Beniwal R, Sharma S, Kochar DK, Tuteja FC, Ghori SK and Sahani MS (2005). Effect of raw camel milk in type 1 diabetic patients: 1 year randomised study. Journal of Camel Practice and Research 12:27-31.

Al Dahouk S, Tomaso H, Nockler K, Neubauer H and Frangoulidis D (2003). Laboratory based diagnosis of Brucellosis - a review of the literature. Part II: Serological tests for brucellosis. Clinical Laboratory 49:577-589.

Alton GG, Jones LM and Pietz DE (1975). Laboratory Techniques in Brucellosis. WHO Monograph Series No. 55, 2nd Edn, WHO Geneva.

Andreani E, Prosperi S, Salim AH and Arush AM (1983). Serological and bacteriological investigation on brucellosis in domestic ruminants of the Somali Democratic Republic. Revue D'élevage et de Médecine Vétérinaire Des Pays Tropicaux 35:329-33.

Baily G, Krahn JB, Drasa BS and Stokeer NG (1992). Detection of *Brucella melitensis* and *Brucella abortus* by DNA

amplification. Journal of Tropical Medicine and Hygiene 95:271-275.

Blaizot C (1976). Etude des parasitoses gastro-intestinales du dromadairedans la région de Dire' daoua (Sud-estéthiopien) etessaisthérapeutiques. Doctoral thesis, Ecole NationaleVétérinaire, Maisons-Alfort, France. 62.

Bougnoux ME, Dupont C, Mateo J, Saulnier P, Faivre V, Payen D, Nicolas-Chanoine MH (1999). Serum is more suitable than whole blood for diagnosis of systemic Candidiasis by nested PCR. Journal of Medical Microbiology 37: 925.

Brown PD, Gravenkamp C, Carrington DG, Van de Kemp H, Harrrtskeerl RA, Edwards CN, Everard CO, Terpstra WJ and Levett PN (1995). Evaluation of Polymerase chain reaction by early diagnosis of Leptospirosis. Journal of Medical Microbiology 43:110.

Cooper CW (1991). The epidemiology of human brucellosis in a well-defined urban population in Saudi Arabia. Journal of Tropical Medicine and Hygiene 94:416-422.

Gameel MA, Mohamed OS, Mustafa AA and Azwai MS (1993). Prevalence of camel brucellosis in Libya. Tropical Animal Health and Production 25:91-93.

Gupta VK, Ranjeeta K, Jyoti Vohr, Vihan VS (2009). Comparison of serum and whole blood PCR for diagnosis of goat brucellosis. The Indian Veterinary Journal 86:335-338.

Gwida M, Gohary AE, Melzer F, Khan I, Rösler U and Neubauer H (2012). Brucellosis in camels. Research in Veterinary Science 92:351-355

Hamada S, EL-Hidik M, Sherif I, EL-Sawale H and Yousef M (1963). Serological investigation on brucellosis in cattle, buffaloes and camels. Journal of Arabian Veterinary Medical Association 23:173-178.

Kawamura S, Maesaki S, Noda T, Hirakata Y, Tomono K, Tashiro T and Kohno S (1999). Comparison between PCR and detection of antigen in sera for diagnosis of pulmonary aspergillosis. Journal of Clinical Microbiology 37:218-220.

Mathur KN and Bhargava SC (1979). Seroprevalence of Q fever and brucellosis in camels of Jorbeer and Bikaner, Rajasthan State, India. Indian Journal of Medical Research 70:391-393.

Morata P, Queipo-Ortuno MI, De Dios and Colmenero J (1998). Strategy for optimising DNA amplification in a peripheral blood PCR assay used for diagnosis of human brucellosis. Journal of Clinical Microbiology 36:2443-2446.

Murdoch DR, Walford EJ, Jennings LC, Light GJ, Schousboe MI, Chereshsky AY, Chambers ST and Town GI (1996). Use of the polymerase chain reaction to detect Legionella DNA in urine and serum samples from patients with pneumonia. Clinical Infectious Diseases 23:475.

Musa T, Eisa ZE, Sanousi M, Abdel Wahab B and Perrett L (2008). Brucellosis in Camels (*Camelus dromedarius*) in Darfur, Western Sudan. Journal of Comparative Pathology 138:151-55.

OIE (2009). Manual of Standards for Diagnostic Tests and Vaccines.

Okoh AEJ (1979). A survey of brucellosis in camels in Kano, Nigeria. Tropical Animal Health and Production 11: 213-214.

Radwan AI, Bekairi SI and Prasad PV (1992). Serological and bacteriological study of brucellosis in camels in central Saudi Arabia. Revue Scientific Technic 11:837-844.

Radwan AI, Bekairi SJ, Mukayel AA, Albokmy AM, Prasad PVS, Azar FN and Coloyan ER (1995). Control of Brucella melitensis infection in large camel herd in Saudi Arabia using antibiotherapy and vaccination with Rev.1 vaccine. Revue Scientifique et Technique de l Office International des Epizooties 14:719-732.

Shome R, Gangadar NL, NarayanaRao K, Shome BR and Prabhudas K (2011). Diagnosis of brucellosis in the equines by serological tests and PCR: A clinical report. The Indian journal of Animal Sciences 81:137-139.

Richard B (1975). Etude de la pathologie du dromadairedans la sous-province du Boruna (Ethiopie). Doctoral thesis no. 75, EcoleNationaleVétérinaire, MaisonsAlfort, France, 181.

Solonitsuin MO (1949). Brucellosis in camels. Veterinary, Moscow 26:16-21.

Teshome H, Molla B and Tibbo M (2003). A seroprevalence study of camel brucellosis in three camel rearing regions of Ethiopia. Tropical Animal Health and Production 35:381-90.

Vivekananda, Shome R, Isloor S, Rathnamma D, Veeregowda BM, Suryanarayana VVS, Shome BR and Prabhudas K (2012). A multi-pronged approach for diagnosis of *Brucella melitensis* infection in sheep flocks. Indian Journal of Animal Sciences 82:4-10.

Wernery U and Kaaden OR (2002). Infectious Diseases of Camelids. London, Blackwell Wissenscha

Zerva L, Bourantas K, Mitka S, Kansouzidou A and Legakis NJ (2001). Serum is the preferred clinical specimen for diagnosis of human brucellosis by PCR. Journal of Clinical Microbiology 39:1661-1664.