



Original Research Article

Sexual Transmission of Human Brucellosis: Case StudiesShome R^{1*}, Nagarathna S^{2#}, Prashant G^{2##}, Nagalingam M^{1**}, Padmashree B.S^{##}, Triveni K^{1##},
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ABSTRACT**Background:** Brucellosis continues to be a major public and animal health problem in many regions of the world, particularly where livestock are a major source of food and income. Human cases continue to occur following international travel, traditional use of raw milk products and close contact with infected animals. In livestock, sexual transmission of brucellosis is well documented with *Brucella* being present in both semen and vaginal secretions. In humans, sexual transmission has rarely been reported.**Methodology:** In the present study, serological studies were used to provide quick diagnosis to brucellosis suspected couples. We selected 14 samples from 7 couples (husband and wives) and tested with Rose Bengal plate test (RBPT), Standard tube agglutination test (SAT), indirect ELISA and PCR.**Results:** In RBPT, 5 out of 7 males and 2 out of 7 female were positive. Significant SAT titres was observed in 3 and 2 males and females, respectively and similarly in IgM ELISA, 3 males and 2 female serum samples were positive. Majority of the males (6/7) were positive by IgG ELISA. Among seven couples screened by BCSP31 PCR, amplification of 223bp product was observed in 3 males and 2 females (2 couples).**Conclusion:** Study concluded that brucellosis in human has true potential of sexual transmission and should not be ignored.**Key Words:** ELISA, human brucellosis, RBPT, sexual transmission.**INTRODUCTION**Human brucellosis is a chronic granulomatous zoonosis caused by the facultative intracellular bacteria of the genus *Brucella*. Among the *Brucella* species, *Brucella melitensis* (goats, sheep) *B.suis* (pig) and *B.abortus* (cattle) are the most virulent in humans. Brucellosis continues to be a major public and animal health problem in many regions of the world, particularly

where livestock are a major source of food and income. There are many reasons why brucellosis remains endemic. These include expansion of livestock herds and flocks, with associated uncontrolled movements; lack of veterinary support services and vaccines; and poor husbandry practices favoring the spread of infection. Infection can occur through consumption of infected unpasteurized dairy products, while

handling infected animals/cultures in occupationally exposed groups like farmers, veterinarians, and laboratory and slaughterhouse workers. [1-5]

Different routes of disease transmission to humans include contamination of skin abrasions, inhalation of airborne aerosols from animal manure or bacterial culture and rarely, person-to-person transmission can be transmitted sexually. [2] Although brucellosis is primarily transmitted to humans through the consumption of unpasteurized dairy products contaminated with *Brucella* species, several reports have also indicated that brucellosis transmission from man to female partner. [2] Here, we describe awareness of risk group about sexual transmission of brucellosis and voluntarily reporting to the institution for the diagnosis of brucellosis. Investigation of such cases and probable person-to-person sexual transmission of brucellosis in couple's samples has been described.

MATERIALS AND METHODS

Serum samples: During the course of the study from 2006-2012, a total of 1083 human serum samples were tested in the institute (data not shown) including the samples from 7 couples which are the subject of this study are given in Table I. History of the couples in structured format and consent for collection of blood was obtained. Blood samples with and without anticoagulant were collected in vacutainers and blood samples without anticoagulant was allowed to clot. Serum was separated, centrifuged at 3000 rpm for 5 min and stored at -20°C until use.

Rose Bengal plate test (RBPT) and Standard tube agglutination test (SAT): Serum samples were initially subjected to rapid screening RBPT test according to standard procedures. [3] Briefly, for the RBPT, undiluted serum sample (30µl) was mixed with an equal volume of colored

antigen on a glass slide. The results were rated negative when agglutination was absent and 1+ to 4+ ratings as positive, according to the strength of the agglutination within 1 to 3 min. RBPT positive serum samples were further evaluated by SAT and 2ME SAT (2 β mercaptoethanol) by preparing two-fold serial dilutions of the serum samples starting at a dilution of 1:10 in the test tube and the addition of an equal volume of plain antigen according to Weybridge technique. [3] The 2ME test is identical to SAT except that 2ME was added to each test tube to a final concentration of 0.05 M. The mixtures were incubated for 18-24 hours at 37°C and read by visual inspection for transparency of suspension and mat formation. The highest dilution of the serum which showed 50 percent agglutination was taken as end point titre and titre of 1:160 (320 IU/ml) and above was considered positive for human brucellosis. [4,5] The *B. abortus*S99 colored and plain antigens were procured from Institute of Animal Health and Veterinary Biologicals (IAH&VB), Hebbal, Bangalore, India.

Indirect IgG and IgM ELISA: The polysorp micro titer plates (Nunc, Germany) were coated with 1:300 dilution of smooth lipopolysaccharide antigen (sLPS) at 100 µl per well (10ng/well) in carbonate-bicarbonate buffer (pH 9.6) and incubated 4°C for overnight. Antigen coated plates were washed three times with PBST wash buffer (Phosphate buffered saline containing 0.05 per cent Tween 20) pH 7.2. The convalescent sera positive by RBPT, DOT-ELISA and showing 2ME-SAT titer of 1:640 (1280IU /ml) and SAT titres of 1:1280 (2560IU /ml) were considered positive convalescent sera controls for IgM ELISA and IgG ELISA. Test and control sera diluted in PBST blocking buffer (1:100) containing 2per cent bovine gelatin and was added to respective wells (100 µl) of the plates in duplicates (test sera) and

quadruplicate (controls) and incubated at 37°C for 1 hour. The plates were then washed as mentioned earlier. The anti-human IgG and IgM HRP conjugates (Pierce, Germany), diluted 1:8000 in PBST buffer were added to all the wells (100 µl) and incubated for 1 hour at 37°C on orbital shaker (300 RPM /minutes). After washing, freshly prepared O-Phenylenediamine dihydrochloride (OPD) (Sigma, Germany) solution containing 5 mg OPD tablet in 12.5 ml of distilled water and 50 µl of 3% H₂O₂ was added 100µl each well and kept for color development for 10 minutes. Enzyme-substrate reaction was stopped by adding 1M H₂SO₄ (50 µl) and color development was read at 492 nm using an ELISA micro plate reader (Biorad). The optical density (OD) obtained for the negative and positive samples were interpreted by cutoff values set at 3 standard deviations above the arithmetical mean of the OD obtained for the healthy controls. [6]

Amplification of Brucella genus by PCR:

The genomic DNA was extracted from blood using DNeasy blood and tissue kit protocol (QiAgen, USA). Genus specific primers as per Baily *et al.* (1992) [7] and *B. abortus* S99 procured from National *Brucella* reference Laboratory, Division of Public Health, IVRI, India were used as positive controls. The genus PCR was carried out in 25µl reaction volume with 10µl of template DNA from clinical samples or 2 µl of DNA from cultures (approximately 30ng/ reaction), 0.2µM of B4 and B5 primers with 2X Go Taq green master mix (Promega) and cycled (1 min at 93°C, 30 sec at 60°C, 45 sec at 72°C) 35 times in a thermal cycler (Eppendorf). The products were analyzed by electrophoresis through 1.5 % agarose gel stained with ethidium bromide.

RESULTS

Seven couples voluntarily visited/ sent the samples to the institution presuming and suspicious of brucellosis (Table 1). All the males in the study belonged to risk group [veterinary surgeons (n=5); pig farmer (n=1) and para veterinary staff (n=1)]. Among females, two belonged to risk group (staff nurse and quality control officer in dairy plant) and rest were house wives (n=4) and teacher (n=1)). When symptoms feedback was analyzed, 2 out of 7 males stated pain and swelling in the testicles (orchitis) and intermittent fever by 5 males and 1 female patient. Generalized symptoms of arthralgia and myalgia were expressed by majority of the respondents. Among 7 couples, one stated recurrence of brucellosis, infertility and depression and one woman was pregnant (Table I). In the present study, serological studies were preferred to provide quick diagnosis. In RBPT, 5 males and 2 females out of 7 couples were positive. In SAT, 3 males and 2 females respectively showed significant SAT titres and rest had non-significant titres (Table II). In IgM ELISA, 3 males and 2 females, serum samples were positive. Majority of the males (6/7) and less number of (2/7) females were positive by IgG ELISA. Among seven couples screened by PCR, only in three males and two females, amplification of 223bp product was observed (Fig.1)

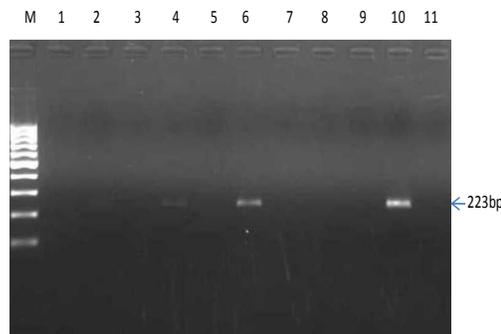


Fig.1. *Brucella* genus specific PCR for human clinical samples. Lane M is 100bp ladder; Lane 1,2,3,5,7,8 & 9 are negative samples, Lane 4 & 6 are positive samples (couple1) ; Lane 10 is positive control, & Lane 11 is negative control DNA (*E.coli*)

Table 1: History of human samples

Couple No	Age in years/sex	Occupation	Symptoms				Treatment obtained For brucellosis or not
			Fever	Arthralgia	Orchitis	Any other symptom/condition	
1	39/M	Veterinary surgeon	yes	yes	yes	sweating	No
	32/F	House wife	-	yes	-	myalgia	No
2	53/M	Para Veterinary staff	yes	yes	yes	chills	yes
	39/F	House wife	yes	yes	-	-	yes
3	57/M	Pig farmer*	yes	yes	-	-	No
	49/F	House wife	-	-	-	headache	No
4	32/M	Veterinary surgeon	-	yes	-	burning of eyes	No
	29/F	Quality control officer in dairy plant	-	-	-	pregnant	No
5	35/M	Veterinary surgeon	yes	yes	-	Infertility, depression	yes
	31/F	School teacher	-	yes	-	bodyache	yes
6	34/M	Veterinary surgeon	Yes	yes	-	-	No
	29/F	Nurse	-	yes	-	-	No
7	37/M	Veterinary surgeon	-	yes	-	anorexia	No
	29/F	House wife	-	yes	-	-	No

M= male; F= female; *handled abortion in pig farms

Table II: Serological and PCR results of the human samples tested for brucellosis

Couple No	Sex	RBPT	SATitre	IgG ELISA	IgMELISA	PCR	Interpretations
1	Male	+	1:640	+	+	+	Recommended for treatment
	Female	+	1:320	+	+	+	Recommended for treatment
2	Male	+	1:320	+	+	+	Recommended for treatment
	Female	+	-	+	+	-	Recommended for treatment
3	Male	+	1:160	+	+	+	Recommended for treatment
	Female	-	-	-	-	-	Negative
4	Male	+	1:40	-	-	-	Retest
	Female	-	-	-	-	-	Negative
5	Male*	+	1:80	+	-	-	Retest
	Female	-	-	-	-	-	Negative
6	Male	-	-	+	-	-	Retest
	Female	-	-	-	-	-	Negative
7	Male	-	-	+	-	-	Retest
	Female	-	-	-	-	-	Negative

NT- Not tested because of non-availability of the blood/ serum sample; *brucellosis recovered patient; + represents positive; - represents negative

DISCUSSION

The clinical picture of human brucellosis is not specific because of intermittent or remittent fever accompanied by malaise, anorexia and prostration or few objective signs are apparent that specifically point to brucellosis.^[4] Because of the deceptive nature of the clinical signs and symptoms of brucellosis, the disease may be easily misdiagnosed and is often diagnosed as pyrexia of unknown origin.^[8,9] Sexual

transmission of brucellosis is frequent feature in livestock and may be underappreciated in humans.^[8] Recently, occasional cases of person to person transmission of brucellosis have been reported in which circumstantial evidence suggests close personal or sexual contact as the route of transmission.^[8]

In present study, we have carefully investigated the cases to establish the transmission of brucellosis through sexual

contact. Among the 7 couples tested, 5 of them have given the samples voluntarily presuming brucellosis. The other couples have referred by clinicians to our institution. With the exposure history to infected animals along with characteristic symptoms such as fever, myalgia, arthritis, recurrent episodes of fever, absence of other viral and bacterial diseases and most importantly not responding the routine antibiotic therapy, the clinicians suspected the possibility of brucellosis.

Accurate diagnosis for brucellosis is made based on the results of two or more tests and blood culture is considered gold standard test. [4] Since, isolation is time consuming; at time it gives inconsistent results in such conditions many cases may be undiagnosed and resulting in unnecessary morbidity. Hence, serological tests are usually preferred for diagnosis of brucellosis rather than isolation because they are inexpensive, fast and simple to perform and with combination of serological test, it is possible even to distinguish stages (active/acute and chronic) of infections. Because of these problems, many laboratories follow initial testing using a screening test like RBPT which has high sensitivity but low specificity. To avoid false positivity results associated with RBPT, the RBPT positive samples are subjected to confirmatory tests such as SAT, ELISA and recently PCR which provide good sensitivity and higher test specificity. [10]

In the study, all the couple samples were subjected to battery of serological tests and PCR in the absence of isolation. In RBPT, 5 males and 2 females out of 7 couples were positive and 3 and 2 males and females, respectively showed significant SAT titres. Multiple tests for detection of antibody classes and antigen along with history and symptoms aid accurate diagnosis of brucellosis. Detection of IgG and IgM

antibody by SAT and ELISA is important in brucellosis for differentiation of acute versus chronic stages of brucellosis. Early in infection, antibodies of IgM class predominate; followed shortly by IgG antibodies due to isotype switching. The IgG antibody has a delayed appearance, although it is found together with IgM four weeks after the initial antigenic stimulus. [4] Only 3 and 2 males and females serum samples respectively were positive by SAT and IgM ELISA, whereas, majority of the males and two females were positive by IgG ELISA. High titer of IgG antibody was a clear indication of chronic phase of the disease or earlier exposure to *Brucella*. Presence of IgM antibodies indicated recent infection and very good supportive clue for the immediate initiation of the treatment.

To confirm presence of genome in the peripheral blood, PCR assay is widely used diagnostic tool and has shown to be a highly specific and sensitivity approaching 100%, even in culture- negative cases. [11] In overall study, one male and two couples were declared positive based on PCR. The probable sexual mode of transmission was confirmed based on serology, PCR, clinical signs and history that the couple never consumed raw milk/ milk products. Both the positive males are associated with veterinary profession and clinical sign arthritis. These female partners were house wives, vegetarians and consuming only pasteurized milk and milk products. In the absence of other risk factors for the disease, sexual route of disease transmission is confirmed. Though, three males were positive in both RBPT and SAT and PCR and only two of the spouses were serologically and PCR positive. The study also clearly indicated some kind of awareness of the veterinarians and staff working in the veterinary hospitals about the risk of disease transmission from infected livestock and possible transmission to their spouse as couple had similar

symptoms. In brucellosis-endemic areas, epididymo-orchitis should also be considering the likelihood of brucellosis and history, physical examination and laboratory evaluation help in diagnosis. [14]

Other modes of transmission reported rarely are infection during pregnancy carries the risk of abortion or intrauterine transmission of infection to the infant. [12] Breastfeeding also may result in transmission to the breastfed infant but this probably is rare. [13] Prospective follow-up of male patients with semen cultures and PCR (regardless of symptoms) may help in assessing the true potential of sexual transmission in future cases. [15-23] On a practical level, abstaining from intercourse or using relevant prophylactic measures during the course of illness should be recommended.

Based on the laboratory findings, history of association with animals and symptoms, the 3 males and two females were recommended for treatment. The 2RBPT males were advised for retesting after 45 days and rest of them were tentatively declared negative for brucellosis. Treatment with combination of antibiotics such as streptomycin for the first 3 weeks with oral rifampicin and doxycycline 6 weeks under medical supervision was recommended. Improvement in the condition like regression of fever, pain and other symptoms were reported by the patients after 6 weeks of treatment. The follow tests after 6 and 12 weeks showed persistence of IgG by RBPT, SAT and ELISA. Whereas, IgM by SAT and ELISA and PCR were negative indicating successful recovery.

The alertness of practitioners and health workers as well as the availability of laboratory facilities for testing are essential for diagnosis of brucellosis.

CONCLUSION

The study reports the diagnosis of human brucellosis by combination of serological s and PCR test and recorded the transmission of brucellosis from infected male to spouse through sex.

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