Original Article

Human brucellosis: Seroprevalence and associated exposure factors among the rural population in Nagpur, Maharashtra, India

Satish L. Ghugey[1], Maninder S. Setia[2], Jyotsna S. Deshmukh[3]

[1]Department of Community Medicine, Mahatma Gandhi Institute of Health Sciences, Mahatma Gandhi Mission University, GM Campus, Sector 1, Kamothe, Navi Mumbai, Maharashtra, [2]Mahatma Gandhi Mission Institute of Health Sciences, Mahatma Gandhi Mission University, GM Campus, Sector 1, Kamothe, Navi Mumbai, [3]Indira Gandhi Government Medical College and Hospital, Central Ave, Mominpura, Nagpur, Maharashtra, India

Abstract

Introduction: Brucellosis is a recognised occupational threat among animal handler and raw animal product consumers. In India, there is a likelihood of missed diagnoses and under-reporting cases by physicians causing an extended debilitating illness. We steered research to conclude the seroprevalence and risk factors allied with Human Brucellosis (HB) among the rural population in Nagpur District, Maharashtra, India. **Methods:** Closed-ended questionnaires used for a cross-sectional study to collect data for demographics and risk exposure variables. 382 subjects' serum-samples were tested by using Rose-Bengal (RBPT) and ELISA technique. An odd ratio calculated for risk factors for HB reported positive or negative. Data were analysed by using SPSS. **Results:** The brucellosis seroprevalence in rural Nagpur was 1.83%. The mean age was 42.32 years, 78.5% were male, and 21.5% were female. Prevalence was higher among males [85.7%] than females [14.3%]. The risk for brucellosis among males (OR = 1.65, 95% CI = 0.19–13.92, $P = 0.64$) was more than females. Handling raw meat had more risk (OR = 3.14, 95% CI = 0.40 – 28.6, $P = 0.23$) than those not handling raw meat. Milking animal was protective (OR = 0.88, 95% CI = 0.80 – 0.96, $P < 0.001$) for brucellosis than those not milking animal. Subjects reported more likely to be a seropositive to human brucellosis those involved in assisted animal delivery ($P = 0.001$), drinking unpasteurised milk ($P<0.001$), consuming milk products made from raw milk ($P<0.001$) and eating raw meat ($P = 0.001$) **Conclusion:** Health education program is essential to generate awareness for brucellosis in the rural community to prevent animal to human disease transmission.

Keywords: Brucellosis, India, Risk factors, Seroprevalence

Introduction

Brucellosis is zoonotic diseases. It has public health significance, and a neglected animal disease has been eliminated in many industrialised nations.[1] The disease has a consequential impact on both animal and human health as well as tremendous socio-economic influence in developing countries where rural population relies for income mainly on livestock breeding and dairy products.[2-4]

It is an important human disease in the Mediterranean countries of Europe, Middle East, South and Central Asia, Africa, Central and South America. Nevertheless, it is often unrecognised and frequently goes unreported.[5-7]

Brucellosis global incidence varies widely from < 0.01 to > 200 per 1,00,000.[8] In India, human brucellosis prevalence has variation

Address for correspondence: Dr. Jyotsna S. Deshmukh, Indira Gandhi Government Medical College and Hospital Central Ave, Mominpura, Nagpur, Maharashtra - 440018, India.
E-mail: jyotsnan1967@gmail.com

Received: 11-06-2020 **Revised:** 08-09-2020
Accepted: 22-09-2020 **Published:** 27-02-2021

Access this article online

Quick Response Code:

Website: www.jfmpc.com

DOI: 10.4103/jfmpc.jfmpc_1153_20

How to cite this article: Ghugey SL, Setia MS, Deshmukh JS. Human brucellosis: Seroprevalence and associated exposure factors among the rural population in Nagpur, Maharashtra, India. J Family Med Prim Care 2021;10:1028-33.

from the lowest 0.8% in Kashmir to 26.66% in Ludhiana.[9,10] Brucellosis is endemic in livestock in the Indian Subcontinent, including India.[11] It is an established endemic disease in cattle population with a prevalence of 1.8% in 19 of 23 states in Indian Subcontinent (1998) and 24.3% in India (2005).[12,13]

Several types of Brucella that are significant to public health exist amongst which B melitensis and B suis are added virulent for humans than B abortus and B canis. However, serious complications can arise through any species of Brucella.[14] Persons get infected either by direct contact through blood, placenta or uterine secretions of infected animals, through interruptions in the skin, by inhalation or by consumption of raw milk and other dairy products.[15,16]

Almost 69% of the Indian population lives in rural areas (Census 2011).[17] Most of them have a close interaction with domestic animals due to their occupation, mostly farming. Hence, they have an amplified hazard of contracting several zoonotic illnesses, including brucellosis.[11]

This study focuses on the rural population because brucellosis is a documented occupational risk infrequently detected in the most health care services, including Primary Health Care [PHC] services, in rural India because of limited laboratory capacity.[18] Therefore, to know the true seroprevalence of human brucellosis, a physician in PHC would prescribe qualitative Rose Bengal Test [RBT], which is easy to process and cheaper. Subsequently, a physician in PHC can educate the patient about brucellosis prevention strategy so that community will become aware.

Next, brucellosis is not included as a priority communicable disease under the Integrated Disease Surveillance Program [IDSP], a public health surveillance and response system, in India.[19] Not more studies have been done to assess the prevalence of brucellosis in the rural population of India.

Aim of this study is to know seroprevalence, and associated exposure factors among the rural population in Nagpur, Maharashtra state of India to provide reference line evidence as well as give indications about the scope of the problem and intervention needs in the study area.

Materials and Methods

Study site and design

From October 2016 to January 2018, a cross-sectional study was conducted by selecting a study population in 15 villages in Umrer block of Nagpur district of Maharashtra state, India. Approval from the ethics committee is obtained. Date of the approval is 8th January 2016.

Inclusion criteria: (1) Person was a resident of the selected village for more than six months. (2) Subjects has a history of animal contact or consuming animal products. (3) Subject willing to participate in a research study and sign for written consent. (4) 18 years and above individual presented in the village at the time of screening and interview.

Exclusion criteria: (1) Subject was the resident of selected villages for less than six months, (2) Pregnant lady, (3) History of antibiotic intake in last two months were calculated from the date of screening and interview, (4) Age of the subject less than 18 years, (5) Subject not willing to participate in the research study**.**

Definition

(1) A seropositive individual defined as a subject whose serological sample after screening reported for the presence of Brucella antibodies had a positive brucellosis serological outcome whichever either by Rose Bengal Plate Test (RBPT) or Enzyme-linked Immunosorbent Assay (ELISA).

(2) A seronegative was defined as an individual was any person, residing in the same village, and whose serum was collected at the same time with the seropositive individual and on screening who reported serological result negative for brucellosis either by RBPT or ELISA**.**

Sampling method and recruitment

We did stratified random sampling to select the study subjects. From selected three study blocks, 15 villages selected randomly; 5 villages from each block. Health camps were arranged to collect a blood sample. An eligible individual screened for brucellosis lab tests RBPT and human ELISA IgG test. The unique identification number was given to each screened subject to avoid duplication.

Justification of sample size

For the rural population, the sample size was determined with considering 8.5% known prevalence in West part of India and assuming an expected 4.5% prevalence in the study population in the study area with alpha = 0.0500, power = 0.8000, delta = 0.0400, p0 = 0.0450, pa = 0.0850, with 10% loss to follow up, required sample size was 382.

Questionnaire development and data collection

For the collection of data, all authors' elaborated close-ended questionnaires consisted of two sections. The first section included sociodemographic data question to collect information on age, sex, marital status, education level. The second section for respondent's risk factors for brucellosis level included questions on the mode of disease transmission from animal to human, daily practice for the animal and the handling the animal products, and respondent's food habits.

At first, this questionnaire was developed in English by all authors, and later on, it translated into local language Marathi by the specialists. A questionnaire was pre-tested to allow for refinement by 40 subjects at the first two villages located in Umred Taluka of Nagpur districts, Maharashtra, India. Further,

it revised according to feedback received from the pre-test. Eligible subjects were selected randomly, and informed consent was obtained from them. All subjects had explained the purpose and method of study. Before data collection; it was assured that all subject meeting eligibility criteria for data and sample collection. All interviews were executed verbally at the time of sample collection from the subjects.

Institutional and Ethical considerations

Mahatma Gandhi Mission Institute of Health Science, Navi Mumbai provided permission to conduct this study. This study was approved for ethical consideration by the Research Ethics Committee, Mahatma Gandhi Mission Institute of Health Science, Navi Mumbai. From each eligible subject, informed consent was obtained before the questionnaire administration interview and sample collection.

Laboratory analysis

Collection of serum samples from subjects: Three millilitres (ml) of intravenous blood was collected into sterile 5 ml plain serum tube by trained health workers. A collected sample in-plane syringe kept in a slanted position on the ice. Infection prevention measures were taken at the time of sample collection. Clear sera were collected in sterile vials. It was labelled with a unique identification number and stored in a freezer at minus (-) 20°C until sample proceeded for a result.

Serological testing of rural population: Each serum sample was labelled with a code that corresponded to the study site and subject's unique identification number. RBPT specific test was done for each sample and also was screened for Brucella antibodies. The standard protocol mentioned in the published in 2009 Terrestrial Manual was used to perform laboratory test.[20]

Concisely described as per 2009 Terrestrial Manual guideline, serum samples and antigen were brought to room temperature (22 ± 4°C). Around 25 µl of each serum was poured on white tile, and the equivalent bulk of antigen put near each serum spot. Serum and antigen were mixed meticulously using a hygienic wooden bar and read for agglutination proximately after 4 minutes. The agglutination process outcome was noted as positive (+) or negative (-) conditional on whether there were agglutinations or not. Next, screening of sera from the rural population was carried out using human IgG ELISA kits. Lastly, the results were compared to those of the RBPT test conducted before. The reagents in the kit were reconstituted to implement lab test. The test technique was processed according to manufacturers' guidelines. Subjects were reported as positive depend on a positive RBPT or ELISA outcome.

Statistical analyses

Data recorded in questionnaires were entered in Microsoft Excel sheet [Version 2013] and analysed by Statistical Package for Social Science Version 16.0 English software. Demographic variable for seropositive and seronegative output presented in frequency tables. Odds ratio were calculated for associated risk factors along with 95% confidence interval with statistical significance set at $P < 0.05$.

Results

Of the 382 subjects we screened, 300 (78.5%) were male, 82 (21.5%) were female and their ages ranged from 18 to 90 years, the standard deviation of 14 years. The majority of the subjects tested were within the ages 25-55 year. Age group 30-42 was tested positive to the brucellosis tests, and the age group below 30 years and above 42 years were tested negative either by RBPT or human ELISA IgG test. 5 (1.3%) of the subjects screened were positive for human brucellosis using RBPT. Next, 7 (1.83%) of the subjects were positive for brucellosis with human IgG ELISA test. A total of 7 individuals tested positive to at least one of the tests giving an overall seroprevalence of 1.83%. Of 382 subjects screened, 62 (16.2%) had no education, 155 (40.6%) had primary education, 142 (37.2%) had secondary education while 23 (6.0%) had tertiary education. Of the seven subjects who tested positive to RBPT or ELISA, 6 (85.7%) were male, and 1 (14.3%) was female, and all of them were married. Subjects who had lower levels of education were more seropositive to human brucellosis comparatively to those who had secondary and tertiary education [Table 1].

Subjects who were involved in milking animals were also more likely to be seropositive to human brucellosis ($P<0.001$) [Table 2]. The individual was involved in assisted animal delivery were significantly more likely to be seropositive to human brucellosis ($P = 0.001$). Subjects who had food exposure like drinking unpasteurised milk were more likely to be seropositive to human brucellosis ($P <0.001$) as well as among those who were drinking milk products made from raw milk ($P < 0001$). Individuals who were eating raw meat were more likely to be seropositive to human brucellosis ($P = 0.001$).

Table 1: Demographic characteristic of seropositive and seronegative among the rural population in Nagpur district of Maharashtra state, India (*n*=382)

Characteristics	Seropositive individuals *n* [%] =7[1.83]	Seronegative individuals *n* [%]=375 [98.17]
Gender		
Male	6 [85.7]	294 [78.4]
Female	1 [14.3]	81 [21.6]
Marital status		
Married	7 [100]	345 [92]
Unmarried	0 [0]	30 [8]
Education level		
None	2 [28.6]	60 [16]
Primary	4 [57]	151 [40.3]
Secondary	1 [14.3]	141 [37.6]
Tertiary	0 [0]	23 [6]

Table 2: Bivariate analysis of factors associated with human brucellosis in the rural population in Nagpur district of Maharashtra state, India [*n*=382]

Variables	Seropositive *n* [%]	Seronegative *n* [%]	OR [95% CI]	*P*
Work exposure				
Handling aborted fetus	0 (0)	27 (27.2)	1.02 (1.11-1.01)	0.46
Slaughtering animal[#]	7 (100)	15 (4)	1.01 (1.00-1.03)	0.59
Milking animal	7 (100)	54 (14.4)	0.88 (0.80-0.96)	<0.001
Handled raw meat	6 (85.7)	1 (14.3)	3.14 (0.40-28.65)	0.23
Assisted animal delivery[$]	7 (100)	148 (39.5)	0.95 (0.92-9.8)	0.001
Being male worker [gender]	6 (85.7)	394 (78.4)	1.65 (0.19-13.92)	0.64
Food exposure				
Eating raw meat	4 (57.1)	347 (92.5)	0.10 (0.23-0.50)	0.001
Drinking unpasteurised milk[©]	7 (100)	75 (20)	0.91 (0.85-0.97)	<0.001
Drinking milk products made from raw milk	3 (42.9)	356 (94.9)	0.40 (0.00-20)	<0.001

[#]Odd ratio for cohort brucellosis IgG test negative because 100% of cases reported seropositive for those exposure factors. [$]Odd ratio for cohort brucellosis IgG test negative because 100% of cases reported seropositive for those exposure factors. [©]Odd ratio for cohort brucellosis IgG test negative because 100% of cases reported seropositive for those exposure factors

Discussion

Our findings show that prevalence of human brucellosis was 1.83%; this finding is equivalent to the finding of studies done in India with the prevalence of 1.6% (2004) and 1.8% (2008).[21,22] Our finding revealed human brucellosis infection among rural population similar to the finding a study conducted in Pakistan, and India indicated that the dwelling of individuals in a rural community was a risk factor for Brucella seropositivity in humans.[23,24]

The factors associated with seropositivity for brucellosis were assisted animal delivery, drinking unpasteurised milk, drinking milk products made from raw milk and eating raw meat. With these mentioned significant risk factors, males appeared to be more at risk of infection with brucellosis. However, it should be noted that assisted animal delivery and milking animals are male-dominated actions. Thus, it may have accounted for this result.

We found that consuming raw meat ($p < 0.001$) was associated with acquiring human brucellosis in subjects. This result was analogous to the outcomes of studies done in Central Sudan and Nigeria.[7,25]

A parallel study conducted in Saudi Arabia also stated that eating raw meat was a risk factor for mounting brucellosis among families that taking care of animals.[26] Eating habits, along with close contact with infected animals, are elements obligatory to spread brucellosis in man.[27]

We found that subjects acquire infection through consumption of unpasteurised milk ($p < 001$), drink milk products made from raw milk ($p < 0.00$) and eat undercooked meat. A similar finding has been documented from the studies done in Gazza strip Palestine, and Libya.[28,29]

In our study, we found no statistically significant relationship between acquiring human brucellosis and handing aborted foetus ($p = 0.46$) and slaughtering an animal ($p = 0.59$) and handling raw meat ($p = 0.23$). Nevertheless, we will still emphasis the importance of handing aborted foetus, slaughtering an animal, in addition to handling raw meat because the outcome may have been due to low percentage of subject participated in this study were exposed to these three risk factors. In our study, subjects slaughtered animal reported brucellosis negative by RBPT and ELIZA. On the other hand, studies conducted in rural North Tanzania and Chad reveal that brucellosis in humans was strongly associated with handling aborted foetuses and placenta of infected animals.[30,31]

Our findings showed that seropositivity to human brucellosis was higher among subjects who were involved in milking animals ($p < 0.001$). A similar study conducted in Eastern Sudan supported this finding that close interactions with animals at the time of milking animal is a risk factor for developing the disease in the human population.[32]

The limitation of our research was that we did not include subjects less than 18 years of age, who were a portion of the population of subjects in rural Nagpur district of Maharashtra state in India because they were not qualified to give informed consent. Therefore, our results cannot be generalised to the whole population of subjects in Nagpur district of Maharashtra state and entire India. Moreover, as our study was a cross-sectional, causation of brucellosis among subjects cannot be proven.

The subjects were likely infected with brucellosis in the study population area, an endemic area, where they were living especially among livestock. It is also possible that some could have been exposed to the risk factors for brucellosis outside study area when they left their resident place for any reason.

Conclusion

Brucellosis is a public health issue amongst the rural population in the Nagpur city of Maharashtra state, with the low seroprevalence. Majority seropositive were male. Occupational risks practices of significance include assisted animal delivery, handle the raw mutton, and milking the animal. Similarly, food

habits of significance include drinking raw milk, consume raw meat, drinking dairy products made from raw milk.

IDSP, India should focus on strengthening the zoonotic disease surveillance and integrating some major zoonosis into current monitoring and surveillance system on the context of One Health program for prevention, detection and response to emerging and re-emerging diseases. Further, expansion of core interdisciplinary is essential for solid One Health-based effort counter to this illness.

Education activities in the rural community are essential for brucellosis to prevent animal to human disease transmission. Its component should involve (1) mode of disease transmission, (2) not to drink unpasteurised milk and milk products made from it, (3) not to eat raw meat, and (4) use of personal protective devices while assisting animal delivery or birth product. Sensitisation of the rural population and animal caretakers of livestock to vaccinate an animal, its economic output, and the health benefit is crucial footstep in control of brucellosis.

Furthermore, availability of RBPT, an easy to use and simple to perform predominantly within limited health infrastructure in the rural area especially at PHC, would be used for screening in suspected brucellosis cases to know the true seroprevalence of brucellosis.

What is known about this topic?

- Brucellosis is a zoonotic disease that caused risk to the human being and livestock in India;
- This disease can be the source for long term overwhelming illness in human being.

What this study adds?

- There is low seroprevalence (1.83%) among the rural population of Nagpur district in the Maharashtra state of India;
- We identified milking the animals, drinking unpasteurised milk, assisting animal delivery as significant risk factors among rural population in a rural area of Nagpur district in the Maharashtra state of India.

Acknowledgements

We are very thankful to Dr Vijay L Ghugey, Nisarg Vidnyan Mandal, Nagpur for their support in this research to organize health education program and health camps in Umrer Taluka of Nagpur district, Maharashtra, India. The author would resemble to prompt their gratefulness to the subjects who participated in this study.

Was Ethics Committee Approval obtained for this study?
Yes

Was informed consent obtained from the subjects participated in this study?
Yes

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Geering WA, Forman AJ, Nunn MJ. Exotic Diseases of Animals. Australia Government Publishing Service, Canberra, Australia. 1995; 1: 301-306. Available from: https://trove.nla.gov.au/work/21854829.
2. Roth F, Zinsstag J, Orkhon D, Chimed-Ochir G, Hutton G, Cosivi O, *et al.* Human health benefits from livestock vaccination for brucellosis: A case study. Bull World Health Organ 2003;81:867-76.
3. Lindahl JF, Vrentas CE, Deka RP. Brucellosis in India: Results of a collaborative workshop to define One Health priorities. Trop Anim Health Prod 2020;52:387-96.
4. Etemadi A, Moniri R, Saffari M, Akbari H, Alamian S, Behrozikhah AM. Epidemiological, molecular characterisation and risk factors of human brucellosis in Iran. Asian Pac J Trop Med 2020;13:169-75.
5. Abou AE. Fifty years of veterinary public health activities in the Eastern Mediterranean Region. East Mediter Health J 2000;6:796-807.
6. Hoover DL, Friedlander AM. Brucellosis. In: Zajtchuk R, editor. Text Book of Military Medicine: Medical Aspects of Chemical and Biological Warfare. Vol 2. Washington DC, US Department of the Army: Surgeon General and Borden Institute; 1997. p. 513-21.
7. Igawe PB, Okolocha E, Kia GS, Irmiya IB, Balogun MS, Nguku P. Seroprevalence of brucellosis and associated risk factors among abattoir workers in Bauchi State, Nigeria, Pan Afr Med J 2020;35:33.
8. Mantur BG, Amarnath SK. Brucellosis in India-A review. J Biosci 2008;33:539-47.
9. Kadri SM, Ruksana A, Laharwal MA, Tanvir M. Seroprevalence of brucellosis in Kashmir (India) among patients with pyrexia of unknown origin. J Indian Med Assoc 2000;98:170-1.
10. Yohannes M, Gill JP. Seroepidemiological survey of human brucellosis in and around Ludhiana, India. Emerg Health Threats J 2011;4:7361. doi: 10.3402/ehtj.v4i0.7361.
11. Pandit DP, Pandit PT. Human Brucellosis: Are we neglecting an enemy at the backyard? Review article. Med J Dr D Y Patil Vidy 2013;6:350-8.
12. Isloor S, Renukaradhya GJ, Rajasekhar M. Serological survey of bovine brucellosis in India. Rev Sci Tech 1998;17:781-5.
13. Dhand NK, Gumber S, Singh BB, Aradhana, Bali MS, Kumar H, *et al.* Study on the epidemiology of brucellosis in Punjab (India) using Survey Toolbox. Rev Sci Tech 2005;24:879-85.
14. World Health Organization. Brucellosis in humans and animals. 2006. Available from: http://www.who.int/csr/resources/publications/Brucellosis.pdf. [Last accessed on 2010 May 03].
15. Halling M, Young J. Brucella. In: Hui YH, Gorham JR, Murrell KD, Cliver DO, editor. Foodborne Disease Handbook – Disease caused by Bacteria. 2nd ed.. New York:

Marcel Dekker, Inc; 1994. p. 21-70.

16. Aworh MK, Okolacha E, Kwaga J. Human brucellosis: Seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria-2011. Pan Afr Med J 2013;16. Available from: https://pubmed.ncbi.nlm.nih.gov/24876892/.
17. Chandramouli C. Rural-urban distribution of population-provisional population totals. Census of India 2011, Ministry of Home Affairs, India, New Delhi: 15th July 2011. Available from: https://censusindia.gov.in/2011-prov-results/paper2/data_files/india/Rural_urban_2011.pdf.
18. Jamir T, Laskar SA, Sarma V, Deka NN. Brucellosis in patients with pyrexia of unknown origin in Assam, India: A retrospective record review. Lancet Glob Health 2020;8:1-46.
19. Integrated disease surveillance Project [Homepage on the internet], Government of India. Available from: https://idsp.nic.in/showfile.php?lid=3923.
20. OIE Terrestrial Manual. Bovine Brucellosis: 2009; chapter 2.4.3. Available from: http://www.oie.int. [Last accessed on 2010 Jul 25].
21. Mantur BG, Akki AS, Mangalgi SS, Patil SV, Gobbur RH, Peerapur BV. Childhood brucellosis – A microbiological, epidemiological and clinical study. J Trop Pediatr 2004;50:153–7.
22. Mantur BG, Biradar MS, Bidri RC, Mulimani MS, Veerappa K, Kariholu P, *et al*. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. J Med Microbiol 2006;55:897–903.
23. Aher AS, Londhe SP, Bannalikar AS, Mhase PP, Dighe VD. Detection of brucellosis in occupationally exposed humans by molecular and serological techniques. Ind J Comp Micro Immun Infect Dis 2011;32:36-40.
24. Kumar A. Brucellosis: The need of public health intervention in rural India. Prilozi 2010;31:219-31.
25. Mohammed MG. Brucellosis in the Gezira area, Central Sudan. J Trop Med Hyg 1989;2:86–8.
26. Al-Sheban N. Brucellosis in an urban setting. Saudi Epid B 1994;1:1.
27. Jaber L, Dahan S, Haran I. Control of brucellosis in Talbe multi-central collaboration. Harefuah 1999;137:454-6, 511, 510.
28. Awad R. Human brucellosis in the Gaza Strip, Palestine. East Mediterr Health J 1998;4:225-33.
29. Ahmed MO, Elmeshri SE, Abuzweda AR, Blauo M, Abouzeed YM. Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006 – January 2008. Euro Surveill 2010;15:19625. Available fro m: https://pubmed.ncbi.nlm.nih.gov/20684813/.
30. Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. Prev Vet Med 2003;61:279-93.
31. John K, Fitzpatrick J, French N, Kazwala R. Quantifying risk factors for human brucellosis in rural Northern Tanzania. PLoS One 2010;5:e9968.
32. El-Ansary H, Mohammed A, Hamad R, Karom G. Brucellosis among animals and human contacts in eastern Sudan. Saudi Medl J 2001;22:577-9.