Original Article

Seroprevalence and Factors Associated with Risk of Human Brucellosis among Febrile Patients Attending Health-Care Facilities in Bauchi, North-Eastern Nigeria

Hafiz Halilu, Fatima J. Giwa[1], Saad A. Ahmed[2], Mu'awiyyah B. Sufiyan[3], Amina I. Mohammed[4]

Nigeria Field Epidemiology and Laboratory Training Program, Abuja, Departments of [1]Medical Microbiology, [2]Pathology and [3]Community Medicine, Ahmadu Bello University, Zaria, [4]Department of Medical Microbiology, Aminu Kano Teaching Hospital, Kano, Nigeria

Abstract

Background: Brucellosis is a neglected underdiagnosed zoonotic disease of worldwide distribution with varying symptoms similar to those occurring in other febrile illnesses. A recent screening survey conducted among butchers in Bauchi state reported a high prevalence of human brucellosis; however, its burden among patients with other febrile illnesses is unknown. We determine the seroprevalence and factors associated with risk of brucellosis among febrile patients attending health-care facilities (HCFs) in Bauchi metropolis, North-Eastern Nigeria. **Methodology:** We conducted a hospital-based descriptive cross-sectional study of 382 participants using a multistage sampling technique. *Brucella* antibodies were detected using Rose Bengal plate test, and a questionnaire was used to identify risk factors associated with human brucellosis. Data analyses were conducted using Epi Info version 7.0. Bivariate and multivariate analyses were conducted at $P \leq 0.05$. **Results:** Fifty-seven (14.9%) of the 382 febrile patients tested had *Brucella* antibodies. Males: female ratio was 1.1:1, and the mean age was 29.9 (standard deviation ± 12.03). Age, sex, occupational status, keeping animals, having ever milked an animal, handling animals during birth, and consuming unpasteurized milk or meat were found to be significantly associated with brucellosis on bivariate analysis. **Conclusions:** Up to 14.9% of the febrile patients attending HCFs in Bauchi metropolis had *Brucella* antibodies. Age, occupational status, consuming unpasteurized milk or meat, handling animals during birth, and lack of awareness on zoonosis remained independent predictors for brucellosis. Therefore, conducting routine serological screening tests for *Brucella* antibodies in all febrile patients presenting to HCFs will assist in its diagnosis and proper management.

Keywords: Brucellosis, febrile patients, North-Eastern Nigeria, prevalence, risk factors

Received on: 30-06-20 **Review completed on:** 09-07-20 **Accepted on:** 09-09-20 **Published on:** ***

Introduction

Brucellosis is a neglected, underdiagnosed zoonotic disease of worldwide distribution. It can cause severe illness in humans and substantial economic losses in livestock production. The disease can be chronic and is capable of affecting any organ or system.[1] *Brucella* can be transmitted to humans in several ways including the consumption of unpasteurized dairy products, inhalation of the microorganisms as well as transmission through the skin. The disease remained the world's most common bacterial zoonosis for decades. In the year 2015, it was enlisted as the second zoonotic infection of public health concern, with over half a million new cases reported annually.[2]

Access this article online

Quick Response Code:

Website: www.atpjournal.org

DOI: 10.4103/atp.atp_39_20

Brucellosis is predominantly an occupational disease of those working with infected animals or their tissues but can also infect consumers of unpasteurized dairy products and hunters who unknowingly handle infected animals. A study of brucellosis in low- and middle-income countries revealed that ingestion of unpasteurized dairy products and exposure through direct contact with infected animal fluids or tissues, especially the placenta from aborted animals, were the

Address for correspondence: Mr. Hafiz Halilu, Nigeria Field Epidemiology and Laboratory Training Program, Abuja, Nigeria. E-mail: hafeezkh13@gmail.com

How to cite this article: Halilu H, Giwa FJ, Ahmed SA, Sufiyan MB, Mohammed AI. Seroprevalence and factors associated with risk of human brucellosis among febrile patients attending health-care facilities in Bauchi, North-Eastern Nigeria. Ann Trop Pathol 2020;XX:XX-XX.

main risk factors for transmission of the infection.[3] The consumption of local delicacies such as milk products (e.g., cheese and yoghurt) and locally roasted meat/barbecue was found to have a positive relationship with contracting recent *Brucella* infection and increasing the risk of having miscarriage threefold, respectively.[4]

Prevalence rates in some countries are exceeding 10 cases per 100,000 population.[5] The disease is largely occupational, and the majority of the cases are males between the ages of 20 and 45 years.[6] Brucellosis is prevalent in many regions of the world, including Latin America, the Middle East, the Mediterranean Basin, Africa, and Asia.[7] In Northern and sub-Saharan African countries, brucellosis prevalence varies within countries, and the incidence ranges from 0.28 to 71/100,000 populations.[8] In Nigeria, the prevalence of human brucellosis reported by different researchers ranges from 7.6% to 55%, mostly focused on occupationally exposed groups with varying reported results.[9,10]

Brucellosis poses considerable challenges for clinicians in both human and animal health. Clinical signs and symptoms of human brucellosis are nonspecific and highly variable, and these symptoms also occur with common nonzoonotic diseases, such as malaria and typhoid fever, which are likely to be considered more readily by clinicians.[11] There is an increasing report of febrile illnesses across Nigeria and other sub-Saharan African countries, which are not malaria or typhoid fever.[12-14]

A previous study showed that bovine brucellosis is prevalent among cattle slaughtered in Bauchi state.[15] A recent report also revealed a high prevalence of human brucellosis among herders and butchers in the state.[16] It is important, therefore, to determine whether these cases of febrile illnesses are brucellosis or otherwise for proper patient management. We sought to determine the burden of human brucellosis among febrile patients in Bauchi metropolis and its associated risk factors which will help in the proper management of patients with febrile illnesses.

Methodology

Study area

Bauchi state is located in North-Eastern Nigeria. The predominant economic occupation in Bauchi state, especially in rural communities, is agriculture (including livestock keeping) which provides direct and indirect jobs to thousands of people. Bauchi state has a high population of livestock including its domestication and a high rate of consumption of raw/unprocessed milk, which are risk factors for brucellosis.

Study design

This was a hospital-based descriptive cross-sectional study.

Study population

Febrile patients aged 16 years and above presenting to selected health-care facilities (HCFs) and private clinics were enrolled.

Inclusion and exclusion criteria

Febrile patients aged 16 years and above presenting to public tertiary, secondary, primary, or private health facilities (HFs) with temperature of 38°C or more were included. The study excluded patients with known underlying chronic illnesses (cancer, HIV, and tuberculosis patients) and those on admission (inpatients).

Sample size determination

The sample size was determined using the Leslie Kish formula for cross-sectional studies, where a sample size of 335 was obtained using a prevalence of 32.5% from a previous study.[15] After correction for a 20% nonresponse rate, the sample size became 400. A total of 400 patients were recruited for this study, however, only 387 participants consented giving a 96.8% response rate. Furthermore, 387 blood samples were collected, of which only 382 were found to be acceptable for testing.

Sampling technique

A multistage sampling technique was used to select the study participants. There are 11 political wards in Bauchi metropolis and 11 public and 20 private HFs.

Stage 1: Selection of ward

A simple random sampling technique by balloting was used to select five wards from the 11 wards that made up the metropolis. The following wards were selected: Dan-Iya, Hardo, Dan-Amar A, Makama B, and Dan-Dango.

Stage 2: Selection of health facilities from the selected wards

HFs from the selected wards were stratified into public and private facilities before the selection was carried out. They were then selected by equal allocation to public and private facilities; one public and one private health facilities were selected from each selected ward using simple random sampling by balloting, making a total of two facilities from every ward and a total of ten facilities from all the five wards.

Stage 3: Selection of the respondents from the selected health facilities

Based on the patient's flow/attendance in the HFs, the average number of patients with fever seen in the outpatients' departments per facility per day was obtained which ranges from 15 to 30/day, Abubakar Tafawa Balewa University Teaching Hospital (ATBUTH) 30, Specialist Hospital Bauchi 30, Infectious Diseases Hospital Bayara 22, Tashar-Babaye Primary Health Care Centre 24, Yalwa Primary Health Care Centre 15, Ni'ima Clinic 30, Remee Clinic 22, Durum PHCC 22, Al-Manzoor Clinic 22, and Kainuwa Clinic 24. A total of average fever cases seen per day in all the 10 HFs were 241. The sample size was divided between the facilities based on proportionate allocation. Using systematic sampling, every fifth patient was selected from each HF everyday (depending on the average number of the febrile patients seen daily in that facility) among patients presenting with fever till the required sample size was reached. For ATBUTH 50, Specialist Hospital Bauchi 50, Infectious Diseases Hospital Bayara 36, Tashar-Babaye Primary Health Care Centre 40, Yalwa Primary Health Care Centre 25, Ni'ima Clinic 50, Remee Clinic 36, Durum PHCC 36 Al-Manzoor Clinic 36, and Kainuwa Clinic 40.

Data collection

Questionnaire

A pretested, interviewer-administered structured questionnaire with five sections was used to collect data on sociodemographics of the participants (sex, ethnicity, age, residential local government area, and occupational status). Risk factors for brucellosis and other zoonoses were also assessed from the questionnaire.

Sample collection

The samples were collected by trained medical laboratory technicians who were recruited as research assistants. Sterile vacutainers were used to collect 3 ml of blood from each patient aseptically. The skin was cleaned in concentric circles of increasing diameter with 70% ethanol and left to dry.

Three milliliters of blood was drawn from the vein and dispensed in a plain bottle. This was labeled using a unique patient identifier (ID), location, and the date of sample collection and then transported in iceboxes at 4°C to ATBUTH, after which it was spun at 1500 rpm for about 5 min, and the serum was extracted into cryovials and stored at −20°C.

Sample processing

Rose Bengal plate test

The Rose Bengal plate test (IDvet, 310, Rue Louis Pasteur, Grabels, France) was used to detect *Brucella* antibodies. This antigen is a bacterial suspension of *Brucella* stained with Rose Bengal and buffered at pH 3.6 and contains 0.95 g/L of sodium azide. The reagent was obtained from the Veterinary Public Health Laboratory at Ahmadu Bello University Zaria, and the test was conducted according to the manufacturer's instructions. All tests were run using positive and negative controls.

Data analysis

Epi Info version 7 Developed by Centers for Disease Control and Prevention (CDC) and MS Excel 2007 were used for data analysis. Data were presented as frequencies, proportions, and summary measures of statistics in tables. The variables analyzed include sociodemographic characteristics, risk factors, and serology result for brucellosis. Bivariate and multivariate analysis (using logistic regression) was carried out. The confidence level was set at 95%, and $P < 0.05$ was considered statistically significant.

Ethical considerations

Ethical clearance with protocol approval number NRECC/12/05/2013/2018/57 was obtained from the Bauchi State Ministry of Health Operational Research Advisory Committee and ATBUTH Bauchi Ethical Committee with assigned number 0054/2018. Informed and written consent was obtained from the participants before enrollment. Collected data were kept confidential; study ID numbers were used for patient identification to ensure utmost confidentiality.

Results

A total of 400 patients were recruited for this study with a 96.8% response rate; 387 blood samples were collected, of which 382 were found acceptable for testing. The age and sex distribution of the participants is shown in Table 1. Two hundred participants (52.4%) were recruited from government-owned HCFs [Figure 1]. The prevalence of brucellosis was 14.9% among febrile patients attending outpatients' departments from the ten selected HFs in Bauchi metropolis (n = 382). Among males, the prevalence was 19.3% (n = 197), while it was 10.3% in females (n = 185). The study found a prevalence of 26.2% among participants that were aged 35 years and above (n = 130). The prevalence of brucellosis was 20.2% among participants with no awareness on zoonoses. A prevalence of 34.7% was also observed (n = 72) in participants who consumed raw or unpasteurized milk or meat and a prevalence of 62.5% among participants who handled animals during birth (n = 32). A prevalence of 42.9% was also observed among study participants who had ever milked animals (n = 56). The seropositivity of brucellosis was found to be 23.4% (n = 201) among patients who reported to have kept animals (either at home farm or both) [Table 2].

Table 1: Sociodemographic characteristics of the study participants (n=382)

Characteristics	*n* (%)
Age group (years)	
<35	252 (66.0)
35-44	84 (22.0)
>44	46 (12.0)
Sex	
Male	197 (51.6)
Female	185 (48.4)
Ethnicity	
Hausa/Fulani	266 (69.6)
Others	116 (30.4)
Marital status	
Single	158 (41.4)
Married	208 (54.4)
Others	16 (4.2)
Educational level	
Primary	45 (11.8)
Secondary	177 (46.3)
Tertiary	113 (29.6)
Nonformal	47 (12.3)
Occupation	
Student	130 (34.0)
Housewife	76 (19.9)
Civil servant	62 (16.2)
Famer/herder	48 (12.6)
Abattoir worker	15 (3.9)
seller of hides/skin	4 (1.1)
Laboratory worker	3 (0.8)
Veterinarian	1 (0.3)
Unemployed	43 (11.2)
Awareness on zoonosis	
No	173 (45.3)
Yes	209 (54.7)

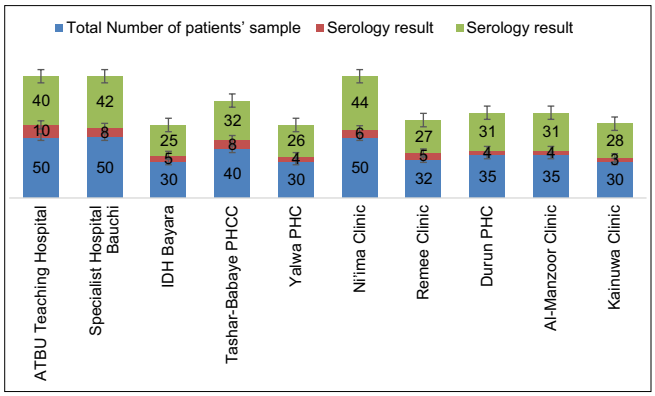

Figure 1: Distribution of study participants selected from different health facilities and their result status

After multivariate analysis using multiple logistic regression, age (being 35 years or younger), low educational level, involving in a high-risk occupation, consuming raw/unpasteurized milk or meat, handling animals during birth, and lack of awareness on zoonoses remained independent predictors for brucellosis among study participants. However, sex, keeping animals, and having ever milked an animal were not statistically significant predictors for brucellosis [Table 2].

Discussion

In this study, the seroprevalence of human brucellosis among febrile patients attending different HCFs in Bauchi metropolis was about 50%. This could be due to previous exposure or current infection. This is lower than reports seen from a similar work which was done in Gombe, Gombe State, North-Eastern Nigeria, which reported 32.5%.[17] Similarly, Ogbodo and Isiofia reported a prevalence of 26.8%[10] among patients tested for malaria in a private medical laboratory in Enugu, south-eastern region of Nigeria. This prevalence for brucellosis in a city not known for pastoral activities is quite high and could be attributed to the serological method used which is known to give false-positive results. The findings of this study were, however, higher than that of another study conducted in Borno State, North-Eastern Nigeria, by Baba *et al.*[18] among patients with pyrexia of unknown origin (PUO) who reported a prevalence of 5.2%. This could be attributed to the fact that Borno State though in the same region is less agrarian due to its unique climatic and weather condition compared to Bauchi state. This indicates that geographical location and other factors like pastoral activities may also influence prevalence. Similarly, Pathak *et al.*[19] reported a prevalence of 4.25% among people with PUO in the Goa region of India. Our study findings were consistent with that obtained in a hospital-based study done in Uganda which reported a prevalence of 13.3% and 17%.[8,19] Another study by Zein and Sabahelkhier[20] in Northern Sudan among high-risk groups also found a prevalence of 15.3%. The prevalence found in this study was, however, lower than the reported prevalence of 40.0% in a study conducted in Libya.[21] The low prevalence of brucellosis found in this study could be attributed to the area of the study which is urban, and brucellosis being a zoonotic disease could be more common in rural areas where keeping animals is one of the common practices among rural dwellers, where methods of rearing animals, hygiene measures, and limited awareness of communities on zoonotic diseases could be more prominent.[22]

We found that a higher prevalence of human brucellosis was influenced by the presence of brucellosis in domestic animals; we demonstrated a higher seroprevalence among patients who kept animals and were five times more likely to develop brucellosis ($P < 0.001$) compared to those who did not which is supported by findings in the Middle East in 2002 by Refai.[22]

In this study, males were two times more likely to develop brucellosis compared to females ($P = 0.001$). This is in concordance with studies conducted in Egypt, Kuwait, Saudi Arabia, and India,[19,23,24] in which males had a higher rate of infection than females, but contrary to findings in South Sudan by Madut *et al.*,[25] who reported that males and females had a similar prevalence of brucellosis. This could be due to the fact that in Muslim communities of Northern Nigeria, females are less involved in agricultural and pastoral activities, but in Southern Sudan communities, females have equal opportunities of handling animals, which potentially exposes them to the same level of risk of acquiring brucellosis as their male counterparts.

The prevalence of brucellosis was higher among patients aged 35 years and above, a finding which corresponds to that of a recent study also by Madut *et al.*[25] in Southern Sudan and that of Kudi *et al.*,[26] which found a high prevalence within the age group of 31–40 years. Individuals within this age group play a major role in rearing animals in addition to performing other tasks such as milking cows and slaughtering of cattle. This could expose such individuals to the risk of acquiring brucellosis, which may be responsible for the high prevalence of the disease among this age group.

The prevalence of brucellosis was also found to be higher among occupationally high-risk groups such as veterinarians, herders, butchers, abattoir workers, and laboratory workers. Using bivariate analysis, these groups were six times more likely to develop brucellosis compared to those in low-risk occupational groups. This could be attributed to their interaction with animals as a result of their occupation which is also in agreement with findings by Aworh *et al*. in Abuja, Nigeria, Zein and Sabahekheier in Northern State of Sudan, and Tumwine *et al*. in Uganda.[9,20,27] The independent predictors for brucellosis using multiple regression analysis were age (35 years or more), low educational level, occupation, consuming raw/unpasteurized milk or meat, handling animals during birth, and lack of awareness on zoonoses.

This could be attributed to direct contact with infected animals or consumption of infected products, which is consistent with the findings of a study by Refai *et al.*[22] which showed that the consumption of unpasteurized dairy products and delivery

Table 2: Risk factors for brucellosis among febrile patients attending health-care facilities in Bauchi metropolis between March and June 2019 (*n*=382)

Characteristics	Brucellosis status		Odds ratio (95% CI)	
	Positive, *n* (%)	Negative, *n* (%)	Crude	Adjusted
Age (in years)				
>35	34 (26.2)	96 (73.9)	2.1 (1.2-3.5)	2.4* (1.1-4.8)
<35	37 (14.7)	215 (85.3)		
Sex				
Male	38 (19.3)	159 (80.7)	2.1 (1.2-3.8)	1.2 (0.5-2.7)
Female	19 (10.3)	166 (89.7)		
Educational level				
Low†	45 (16.7)	224 (83.3)	1.7 (0.9-3.3)	-
High	12 (10.6)	101 (89.4)		
Occupational status				
High risk‡	28 (39.4)	43 (60.6)	6.2 (3.4-11.7)	3.1* (1.4-6.9)
Low risk	29 (9.37)	282 (90.7)		
Keeping animals				
Yes	47 (23.4)	154 (76.6)	5.2 (2.5-8.5)	1.6 (0.5-3.0)
No	10 (5.5)	171 (94.5)		
Consuming raw milk				
Yes	25 (34.7)	47 (27.3)	4.6 (2.5-8.5)	2.3* (1.0-5.0)
No	32 (10.3)	278 (89.7)		
Handling animals during birth				
Yes	20 (62.5)	12 (37.5)	14.1 (6.4-31.1)	6.5* (2.4-17.2)
No	37 (10.6)	313 (89.4)		
Ever milk animals				
Yes	24 (42.9)	32 (57.14)	6.7 (3.5-12.6)	1.7 (0.7-4.1)
No	33 (10.1)	293 (89.9)		
Taking nono/fura and nono				
Yes	47 (16.5)	237 (83.5)	1.7 (0.7-4)	-
No	10 (10.2)	88 (89.8)		
Awareness on zoonoses				
Yes	22 (10.5)	187 (89.5)	2.2 (1.21-3.8)	2.9* (1.4-5.9)
No	35 (20.2)	138 (79.8)		

†Informal, primary, and secondary are low level, while postsecondary is high level, ‡Herders, abattoir workers, veterinarian, butchers, seller of hides and skin, laboratory workers, *Factors that remained significant after multiple logistic regression model analysis. CI: Confidence interval

methods of animals enhanced the spread of the disease. In addition, a study by Omer *et al.*[28] showed that the consumption of raw meat increased the risk of getting infected. Our findings showed that educational level was not an important risk factor which is in concordance with a case–control study conducted by Sofian *et al.* in Iran.[29] It is, however, in contrast with the studies by Njeru *et al.* among febrile patients seeking treatment in remote hospitals in North-Eastern Kenya and Arif *et al.* in Pakistan which shows that low level of education is a significant risk factor for human brucellosis.[30,31] This study shows that lack of awareness on zoonosis in general and brucellosis in particular is an important risk factor for human brucellosis as documented by Arif *et al.* in five districts of Punjab and two districts of Sindh of Pakistan[31] and Mufinda *et al.* in Namibe Province of Angola.[32]

Conclusions

Brucella antibodies from febrile patients attending various health-care facilities in Bauchi metropolis were found to be high (about 15%). Risk factors associated with developing brucellosis were keeping animals and involving in occupations related to handling of animals or their raw products. The major independent predictors for brucellosis in Bauchi metropolis were consumption of infected animal products and animal handling during parturition.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Franco MP, Mulder M, Gilman RH, Smits HL. Human brucellosis. Lancet Infect Dis 2007;7:775-86.
2. OIE. Information on Aquatic and Terrestrial Animal Diseases: OIE-World Organization for Animal Health; 2018. Available from: http://www.oie.int/animal-health-in-the-world/information-on-aquatic-and-

terrestrial-animal-diseases/. [Last accessed on 2019 Mar 30].
3. Shirima GM, Fitzpatrick J, Kunda JS, Mfinanga GS, Kazwala RR, Kambarage DM. Short communications the role of livestock keeping in human brucellosis trends in livestock keeping communities in Tanzania. Tanzanian J Hlth Res 2010;12:203-7.
4. Adesokan HK, Alabi PI, Ogundipe MA. Prevalence and predictors of risk factors for brucellosis transmission by meat handlers and traditional healers' risk practices in Ibadan, Nigeria. J Prev Med Hyg 2016;57:163-71.
5. Pappas G. The changing *Brucella* ecology: Novel reservoirs, new threats. Int J Antimicrob Agents 2010;36:S8-11.
6. Ducrotoy MJ, Bertu WJ, Ocholi RA, Gusi AM, Bryssinckx W, Welburn S, *et al*. Brucellosis as an emerging threat in developing economies: Lessons from Nigeria. PLoS Negl Trop Dis 2014;8:e3008.
7. Gul ST, Khan A. Epidemiology and epizootology of brucellosis: A review. Pakistan Vet J 2007;27:145-51.
8. Dean AS, Crump L, Greter H, Schelling E, Zinsstag J. Global burden of human brucellosis: A systematic review of disease frequency. PLoS Negl Trop Dis 2012;6:e1865.
9. Aworh MK, Okolocha E, Kwaga J, Fasina F, Lazarus D, Suleman I, *et al*. Human brucellosis: Seroprevalence and associated exposure factors among abattoir workers in Abuja, Nigeria-2011. Pan Afr Med J 2013;16:103.
10. Ogbodo SO, Isiofia OP. Co-existence and seroprevalence of brucellosis in malaria-endemic Metropolis in South-Eastern Nigeria. Ournal Exp Res 2016;4:68-73.
11. Prasad N, Murdoch DR, Reyburn H, Crump JA. Etiology of severe febrile illness in low-and middle-income countries: A systematic review. PLoS One 2015;10:e0127962.
12. Dabo NT, Sarkinfada F. Diagnosis of malaria and typhoid fevers using basic tools: A comparative analysis of a retrospective data with a prospective evaluation in an endemic setting. Bayero J Pure Appl Sci 2017;10:448-54.
13. Christopher AE. Aetiologic agents of fevers of unknown origin among patients in Benin City, Nigeria. Malays J Med Sci 2014;21:37-43.
14. Maze MJ, Bassat Q, Feasey NA, Mandomando I, Musicha P, Crump JA. The epidemiology of febrile illness in sub-Saharan Africa: Implications for diagnosis and management. Clin Microbiol Infect 2018;24:808-14.
15. Adamu SG, Atsanda NN, Tijjani AO, Usur AM, Sule AG, Gulani IA. Epidemiological study of bovine brucellosis in three senatorial zones of Bauchi State, Nigeria. Vet World 2016;9:48-52.
16. Alkassim B. Herders, butchers suffer contagious animal disease in Bauchi-Veterinary Expert – Daily Trust; 2018. Available from: https://www.dailytrust.com.ng/herders-butchers-suffer-contagious-animal-disease-in-Bauchi-veterinary-expert.html. [Last accessed on 2019 Oct 03].
17. Jacques G, Axel CJ, Stephan K, David FK, Bruno GJ. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet Res 2005;36:313-26.
18. Baba MM, Sarkindared SE, Brisibe F. Serological evidence of brucellosis among predisposed patients with pyrexia of unknown origin in the north eastern Nigeria. Cent Eur J Public Health 2001;9:158-61.
19. Pathak AD, Dubal ZB, Doijad S, Raorane A, Rodrigues S, Naik R, *et al*. Human brucellosis among pyrexia of unknown origin cases and occupationally exposed individuals in Goa Region, India. Emerg Health Threats J 2014;7:23846.
20. Zein AM, Sabahelkhier MK. Prevalence of brucellosis among high risk groups in Northern State, Sudan. Nov J Med Biol Sci 2015;4:1-4.
21. Ahmed MO, Elmeshri SE, Abuzweda AR, Blauo M, Abouzeed YM, Ibrahim A, *et al*. Seroprevalence of brucellosis in animals and human populations in the western mountains region in Libya, December 2006-January 2008. Euro Surveill 2010;15:19625.
22. Refai M. Incidence and control of brucellosis in the Near East region. Vet Microbiol 2002;90:81-110.
23. Jennings GJ, Hajjeh RA, Girgis FY, Fadeel MA, Maksoud MA, Wasfy MO, *et al*. Brucellosis as a cause of acute febrile illness in Egypt. Trans R Soc Trop Med Hyg 2007;101:707-13.
24. Christopher S, Umapathy BL, Ravikumar KL. Brucellosis: Review on the recent trends in pathogenicity and laboratory diagnosis. Lab Phys 2010;2:55.
25. Madut NA, Nasinyama GW, Muma JB, Sube KLL, Ocan M, Muwonge A, *et al*. Prevalence of brucellosis among patients attending Wau Hospital, South Sudan. Munderloh UG, editor. PLoS One 2018;13:e0199315.
26. Kudi AA, Ahmed RA, Baba-Ali F, Uba A, Tahir F Yusuf IZ. Human brucellosis: Seroprevalence amongst patients attending the general out-patient department (GOPD), federal teaching hospital gombe, Nigeria. Niger Biomed Sci J 2015;8:0794-1056.
27. Tumwine G, Matovu E, Kabasa JD, Owiny DO, Majalija S. Human brucellosis: Sero-prevalence and associated risk factors in agro-pastoral communities of Kiboga District, Central Uganda. BMC Public Health 2015;15:900.
28. Omer MM, Musa MT, Bakhiet MR, Perrett L. Brucellosis in camels, cattle and humans: Associations and evaluation of serological tests used for diagnosis of the disease in certain nomadic localities in Sudan. Rev Sci Tech 2010;29:663-9.
29. Sofian M, Aghakhani A, Velayati AA, Banifazl M, Eslamifar A, Ramezani A. Risk factors for human brucellosis in Iran: A case-control study. Int J Infect Dis 2008;12:157-61.
30. Njeru J, Melzer F, Wareth G, El-Adawy H, Henning K, Pletz MW, *et al*. Human brucellosis in febrile patients seeking treatment at remote hospitals, North-eastern Kenya, 2014–2015. Emerg Infect Dis 2016;22:2160-4.
31. Arif S, Thomson PC, Hernandez-jover M, Mcgill DM, Warriach HM, Heller J. Knowledge, attitudes and practices (KAP) relating to brucellosis in small holder dairy farmers in two provinces in Pakistan. PLoS One 2017;12:1-19.
32. Mufinda FC, Boinas F, Nunes C. Prevalence and factors associated with human brucellosis in livestock professionals. Rev Saude Publica 2017;51:57.