Original Article

Human Brucellosis in Khartoum State: A Commonly Underdiagnosed Disease.

Adam Ahmed Adam Mustafa[1*] and Hassan Sidahmed Hassan[2]

Abstract

Back ground: Human brucellosis is a major debilitating zoonotic disease. It is caused by bacteria of the genus *Brucella*

Methods: The serum antibody titres to *Brucella melitensis* and *Brucella abortus* of one thousand febrile patients, randomly selected from Khartoum, Khartoum North and Omdurman Teaching Hospitals, were estimated by the STAT.

Results: Eighty nine (8.9%) of the febrile patients had brucellosis. The average age of brucellosis patient was 43.9 years. Sixty three (70.8%) of the brucellosis patients were males, and 26 (29.2%) were females. Fifty four (60.7%) of them had significant titres to *Brucella melitensis* while 23 (25.8%) patients had significant titres to *Brucella abortus.* Twelve (13.5 %) patients had significant titres to both *Brucella melitensis* and *Brucella abortus*. The average diagnostic delay of brucellosis in this study was 88.6 days.

Conclusion: Brucellosis was found to be misdiagnosed as malaria or typhoid fever. Animal contact was found to be a significant risk factor.

Keywords: *Brucella melitensis, Brucella abortus,* agglutination.

Human brucellosis is a major debilitating zoonotic disease [1, 2]. It is caused by bacteria of the genus *Brucella*[1, 2]. The disease is endemic in the Sudan and was reported as early as 1908[3]. In spite of this, it is commonly misdiagnosed as another febrile disease[1, 2]. Malaria and typhoid fever are the commonest diseases for which brucellosis is misdiagnosed[4]. The source of any human case is an animal directly or through its raw products[1, 2]. The definitive diagnosis of human brucellosis depends on isolation of *Brucella species* from cultured human specimen. However, these bacteria are slowly growing microorganisms and commonly the culture yields no growth. Even if they grow they are highly infectious to the laboratory personnel[2]. For these reasons, serologic diagnosis has been adopted.

1. Associate Professor in Microbiology, Department of Microbiology, Faculty of Medicine and Health Sciences, Al-Neelain University.
2. Professor in Microbiology, Department of Microbiology, Faculty of Medicine, University of Sciences and Technology.
* correspondence: adamahmed58@hotmail.com
Telephone:0904897829

Materials and Methods

Study Design:

It is a descriptive analytic cross-sectional hospital-based study.

Study Objectives:

The objectives of the study were to determine in the three Hospitals in Khartoum State the:

1. Prevalence of brucellosis.
2. Causative *Brucella* species.
3. Pattern of distribution of brucellosis.

Case definition:

Brucellosis patient was defined as a fever case for at least two weeks with serum antibody titre of 1:160 or more to *Brucella melitensis, Brucella abortus* or to both by the Standard Tube AgglutinatonTest Technique.

Study Population:-

The study population included all febrile patients attending the Medical and Paediatric Clinics in Khartoum, Omdurman and Khartoum North Teaching Hospitals, Sudan.

Sample Size:-

The study sample included a total of one thousand febrile patients randomly selected from these hospitals (400 from Khartoum, 300 from Omdurman and 300 from Khartoum North).

Selection Criteria

a. Inclusion Criteria:

1. Fever for at least two weeks as a main complaint.
2. Willingness of the patient to participate in the study.

b. Exclusion Criteria

1. Patients already diagnosed and on treatment for their fevers.
2. Refusal to participate in the study.

Data Collection

1. Blood Specimens Collection

The skin of the patient at the venepuncture site was disinfected by 70% ethyl alcohol and left to dry. Five millilitres of venous blood were withdrawn by sterile disposable syringe. The blood was left to clot for three hours at room temperature (25 degrees Celsius). Sera were separated from the clotted blood by centrifugation at 3000 rounds per minutes for ten minutes. Each patient's serum was put in a plain sterile plastic container and stored in a refrigerator at + 4 degrees Celsius until tested.

Sera Examination for Antibody Titres

Sera were tested for antibody titres to *Brucella melitensis* and *Brucella abortus* by the Standard Tube Agglutination Test technique (STAT). *Brucella* antigens used for testing sera were purchased from Omega Diagnostics LTD, United Kingdom. In each batch examination positive and negative controls were included. The serum antibody titre was reported from the last tube of the highest dilution showing macroscopic agglutination.

2. Questionnaire administration

Each patient filled in a questionnaire after taking his/her written consent.

The required informations included the age, sex, residence, history of or animal contact and medical history for his/her current disease.

Results

The age of participants ranged between 3- 72 years. 89 patients were seropositive for brucllosis, 63(70.8%) of them were males. B. *melitensis* and B. *abortus* were found in 54 (60.7%) and 23 (25.8%) respectively while both species were found in 12 (13.5 %) patients. Sex distribution was shown in Table1. The average age of brucellosis patient was 43.9 years.

Table (1) showing the 1000 patients ccording to sex and the result of serum antibody titres to *B. melitensis* and *B. abortus*.

Sex	Titre<1:160 (Non-B. cases	Titre≥1:160 B. cases
Males	579	63
Females	332	26
Total	911	89

Sixty one (68.5%) of the 89 brucellosis patients were misdiagnosed as malaria cases and 28 (31.5%) as having typhoid fever.

Sixty three (70.8%) of the 89 brucellosis patients were males, and 26 (29.2%) were females. Seventy seven (86.5%) of the brucellosis patients had history of animal contact.

The average diagnostic delay was 88.6 days.

All the brucellosis patient were improved by medical treatment (Rifampicin and Doxycycline).

The patients who had insignificant serum antibody titres (<1:160) continued with their treating physicians for the full management.

Discussion

The prevalence of brucellosis in this study was found to be 8.9%.

El-ansary et al in Kassala (Eastern Sudan) reported a prevalence of 1% among animal contacts[5]. Musa et al from Nyala in Southern Darfur (Western Sudan) reported a brucellosis prevalence of 18% among febrile patients with history of animal contact[6].

The diagnosis of human brucellosis is usually delayed, because it is commonly misdiagnosed for other febrile diseases[1, 2]. The average diagnostic delay of brucellosis in this study was 88.6 days. The brucellosis patients usually suffer for quite along time before the correct diagnosis is reached, if ever diagnosed. During this period the patients receive unnecessary treatment for non-

existing diseases resulting in prolonged morbidity and unjustified socioeconomic burden. The diagnostic delay of brucellosis was reported worldwide in different countries. In a report from Tanzania the diagnostic delay was found to be about 90 days[7]. Even in a developed country as Germany it was estimated as 75 days[8].

Diagnosis of human brucellosis requires high index of clinical suspicion to the disease in febrile patients with history of contact with animals or their raw products[1, 2]. Such a trend necessitates inclusion of human brucellosis in the differential diagnosis of pyrexia of unknown origin (PUO) in patients with positive history of animal contact.

From the result of tests of sera in this study, *Brucella melitensis* was significantly more common than *Brucella abortus.* High exposure of people to *Brucella melitensis* reservoir may be a reason. Another possible reason for the predominance of *Brucella melitensis* infection over that of *Brucella abortus* was the frequent relapses of the former. Moreover, *Brucella melitensis* has broadened its host specificity. It has been reported that it can infect cattle, camels and horses in addition to its natural hosts, goats and sheep[9]. Such a strain was found to be more virulent and of high resistance to drug treatment[10].

In previous studies in the Sudan, there were no published data that mentioned the species of *Brucellae* that caused human brucellosis except for one study in the Gezira area (Central Sudan), where it was reported that the majority of brucellosis patients (76%) had significant titres to both *Brucella melitensis* and *Brucella abortus* which is different from the 13.5% found in this study and the 40.9% reported elsewhere[11-13].

Such findings were either due to mixed infection or cross reactivity between *Brucella* species and other bacteria. But it is unlikely for cross reacting antigens to result in so high significant titres. In areas where people are intensively exposed to the infecting *Brucella* species, mixed infection is a quite possible explanation. Blood culture and more advanced investigations such as polymerase chain reaction can clarify this debate.

Different studies from other countries also reported the predominance of *Brucella melitensis* over *Brucella abortus* as a cause of human brucellosis. Youssef in a literature review of brucellosis in Saudi Arabia mentioned that *Brucella melitensis* caused 80%-100% of human infections[12].

Sixty three (70.8%) of the brucellosis patients were males. The males to females' ratio were 2.4:1. It was consistent with the finding by K. E. Elbeltagy[13]. Nevertheless, it contradicted the finding by Malik who reported that 61.5% of his patients were females[14]. In two studies; one in Saudi Arabia and the other in Yemen, no significant difference was found between males and females among brucellosis patients[13, 15]. It is not known whether females are naturally more immuned to brucellosis than males or not but males are more exposed to the source of infection.

It was reported that age constituted an important epidemiological risk factor for human brucellosis[12, 16]. The mean age of brucellosis patient in this study was 43.9 years. Sex-wise, the mean age for male patients was 43.9 years while it was 44 years for female patients. There was no significant difference in age between males and females in this study ($p>0.05$). Mahmoud et al in Jordan reported that the majority of their patients were at the range of 34-43 years of age[17]. Their patients were younger than the patients in this study, similar to reports from Yemen.

Forty five (50.6%) of the brucellosis patients were in the age group of 41-60 years. Age is a risk factor in terms of exposure to the hazard of infection. The lowest prevalence of brucellosis in this study was among the patients of 0-5 year's age group, where no brucellosis patient was detected. The low number of brucellosis patients in the younger age group in this study might be due to the late exposure of the people to the hazard of infection.

We found that 86.5% of the brucellosis patients had positive history of animal

contact. This made animal contact a statistically significant risk factor in the epidemiology of human brucellosis ($p<0.05$). Such a finding is consistent with the vast majority of studies on human brucellosis worldwide[1, 2, 4, 8].

Conclusion

The disease was commonly misdiagnosed for other febrile diseases that led to delay in diagnosis. Both *Brucella melitensis* and *Brucella abortus* were found as causes of brucellosis in Sudan. The age at which brucellosis was more prevalent was around 45 years. Animal contact was found to be a major risk factor.

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References

1. Joklik Willett, Amos Wilfert, Wolfgang K et al. *Brucella*, Zinsser Microbiology, 20th edition, McGraw Hill, 1992 609-661.

2. Brooks GF, Butel JS , Morse SA. The *Brucellae*, Jawetz, Medical Microbiology, twenty second edition, McGraw Hill, 2001, 246-248.

3. Dafalla EN. Sudan Medical Records 1928-1937, Sudan: Sudan Records; 1962.

4. Thakur SD,Kumar R,Thapliyal DC. Human brucellosis: Review of an under-diagnosed animal transmitted disease,J Commun Dis. 2002; 34 (4) : 287-301.

5. Elansary HH, Mohammed BA, Abul- Gasim O A. et al. Brucellosis among animals and human contacts in eastern Sudan. Saudi med j, 2000; 22 (7):577-579.

6. Musa M T, Shigidi M T, Saeed O M. Brucellosis in humans associated with animals. Saudi med j 1999; 20(4): 311-315.

7. Kunda J, Fitzpatrick J, Kazwala R et al, Health seeking behavior of human brucellosis in rural Tanzania, BMC Public Health, 2007;7:315.

8. Al Dahouk S, Neubauer H, Hensel A, Schoneberg I, Nockler K, Alpers K, et al, Changing Epidemiology of Human Brucellosis, Germany, 1962-2005, Emerg Infect Dis, Dec 2007, volume 13, no 12, pp1895-1900. http://www.cdc.gov/eid/content/13/12/1895.htm

9. Georgios Pappas, Nikolaos Akritidis, Mile Bosilkovski, Epameinondas Tsianos, Brucellosis (review article) N Engl J Med 2005; 352: 2325-36.

10. M. Monir Madkour, Brucellosis , Madkour's Brucellosis, Second edition, Springer, 2001, p.108- 109.

11. Mohd MG, Brucellosis in the Gezira area, Central Sudan, J Trop Med Hyg 1989 Apr; 92(2):86-88.

12. Youssef A. Al-Eissa, Brucellosis in Saudi Arabia: Past, Present and Future, Annals of Saudi Medicine 1999;19(5): 403-404

13. Elbeltagy, K. E. An epidemiological profile of brucellosis in Tabuk Province, Saudi Arabia, Eastern Mediterranean Health Journal 2001; 7(4/5): 791-798.

14. Malik GM. A Clinical Study of Brucellosis in Adults in the Asir Region of Southern Saudi Arabia. Am J Trop Med Hyg 1997; 56(4):375-377.

15. Al Shamahy H A, Whitty C J, Wright S G. Risk factors for human brucellosis in Yemen: a case control study. Epidemiol-Infect. 2000; 125(2): 309-13.

16. Pappas G, Papadimitriou P, Akritidis N et al. The new global map of human brucellosis. Lancet Infect Dis. 2006 Feb;6(2):91-9.

17. Shehada MN, Odeh JS, Abu-Essud M et al. Seroprevalence of Brucellosis among High Risk People in Northern Jordan. Int J Epidemiol. 1996;25(2):450-4.