ORIGINAL ARTICLE

Human Brucellosis: Incidence, Diagnosis, and Clinical Characteristics of Patients Admitted in a Tertiary Care Setting of Pakistan

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Abstract

Background
Brucellosis is a known widespread zoonotic disease. Humans may be infected with *Brucella* species either due to direct contact with infected animals or ingesting natural products of animal origin. Brucellosis is included in one of the neglected zoonotic diseases, and as per World Health Organization (WHO), almost 500,000 cases are reported every year. The endemicity of this disease in developing countries is likely due to ineffective disease control programs.

Study design
It is a retrospective observational study from the 1988 to 2019.

Results
The study showed the highest incidence of disease was in the 2016 with a male to female ratio of 2.1:1. Species identified were *B. abortus B. melitensis* with positive serological titers of more than or equal to 1:80 or positive blood cultures. The most common symptom in patients was fever.

Keywords
Human; brucellosis; Brucella; *Brucella* species; Pakistan

Introduction

Brucellosis is a known widespread zoonotic disease.[1] Brucella species are gram-negative, facultative intracellular bacilli resulting in a disease with diverse clinical manifestations known as brucellosis. Consumers of unpasteurized dairy products, especially from areas of endemic infection, are at significant risk of food-borne brucellosis.[3, 4]

The clinical presentations of human brucellosis range from non-specific and constitutional symptoms, like prolonged fever, anorexia, or fatigue, to local organ involvement, such as arthritis and neuro-brucellosis.[2] Neurological complications, endocarditis, and testicular or bone abscess formation can also occur.[5]

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Additionally, brucellosis has significant economic ramifications due to time lost by patients from normal daily activities[5] and losses in animal production.[6] Approximately 2% of the untreated patients die of brucellosis.[7]

Four Brucella (B.) genus (*B. abortus, B. melitensis, B. suis,* and *B. canis*) are causative agents of brucellosis in humans. Humans may be infected with Brucella species either due to direct contact with infected animals or ingesting raw products of animal origin.[2]

The World Health Organization (WHO) estimates that 500,000 brucellosis cases occur worldwide annually, 45,000 appear in the Eastern Mediterranean Region. Multiple factors play a role in the spread of disease, including lack of control programs related to zoonotic diseases.[3] The major contributing factor is high livestock demand as a source of income in developing countries.[4]

For every case diagnosed, there are four cases that go undetected.[8] Brucellosis is a major public and animal health problem in many regions of the world, particularly where livestock is a major source of food and income. Despite control programs, it remains endemic in most developing countries.

Material and methods

We conducted a retrospective observational study at Aga Khan University Hospital (AKUH), which is a quaternary care hospital in Karachi, Pakistan.

All patients aged one month and above were admitted with brucellosis as determined by positive serology for *Brucella spp.* (>1:80) or positive blood cultures were included. Patients with a history of tuberculosis, joint infection, meningitis, malignancies were excluded.

The study mainly focused on the incidence of disease in respective years and clinical characteristics of patients admitted. Serological data were also collected.

Data were collected from 1988 to 2019. Microbiological identification was done first by performing gram stain, if gram-negative rods with no growth on McConkey agar (MAC) and growth on sheep blood agar (SBA) further biochemical tests were performed. The bacterium is immotile, oxidase, catalase,

and rapid urease positive. The serological analysis is also performed, the principle is the antigen (stained, killed, and standardized suspensions of) and antibody reaction (antibodies present in patient's serum). Titers are determined by the slide agglutination method.
Medical records of the patients were collected from the HIMS-Health Information Management System department and microbiology laboratory department AKUH. HIMS is an integrated, specialized institution-based information Centre in AKUH. The study was approved by Human Ethics Review Committee, Aga Khan Hospital Karachi.

Covariates included were the patient's demographics, site of infection, and symptomatology of patients.

Statistical analysis: Descriptive analysis was reported as mean and standard deviation (SD) for continuous variables (age) and frequency and percentage for categorical variables (gender, signs and symptoms, serology, and culture). Data analyzed using SPSS software (version 22.0).

Results
There was total of 73 patients admitted with the diagnosis of brucellosis over the last 30 years (1988 to 2019), out of which we found record of 53 patients.

Out of 53 included patients 36 (67.9%) were males and male to female ratio was 2.1:1; with median age of 36 years (Table 1).

The study showed the highest incidence of disease was in the year 2016 (Figure 1). Most patients were residents of Karachi (56%), Sindh Pakistan followed by Baluchistan (23%) (Figure 2). Species identified were *B. abortus, B. melitensis* with positive serological titers of more than or equal to 1:80 (Figure 3). Positive contact history to cattle exposure was present in 8 (15.1%) patients (Figure 4). A positive blood culture in 6 (11.3%) patients (Table 2). The most common symptom was fever 47% (Figure 5).

Discussion
Serological detection of brucellosis is commonly available due to the easy of availability of testing kits and cost effectiveness. Gold standard diagnostic test is detection of Brucella species

Table 1. Demographics of patients

Mean age (Years)	**36.9**
Male	36 (67.9%)
Female	17 (32.1%)
Male: Female	2.1: 1

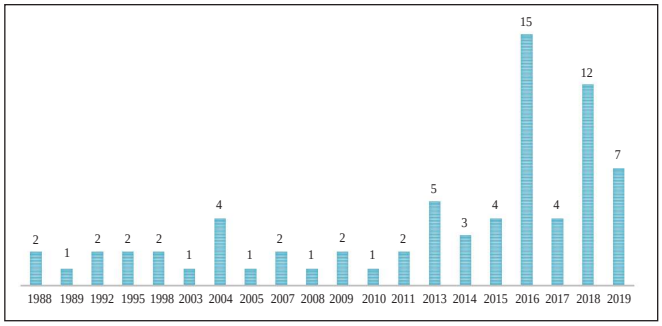

Fig1. Incidence of brucellosis

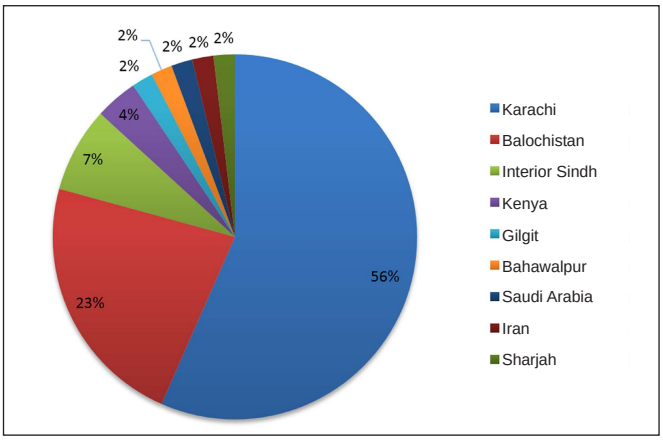

Fig 2. Geographical distribution of patients

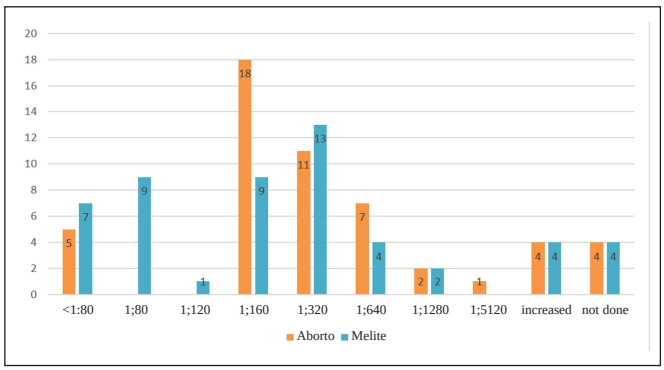

Fig 3. Species serology in patients

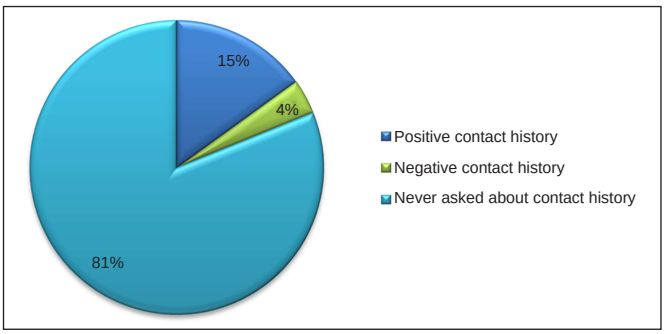

Fig 4. Contact history of patients with livestock/cattle

Table 2: Blood cultures in patients

	Positive	Negative	Positive	Negative
9 (16.9%)	6 (11.3%)	17 (32.1%)	0	21 (39.6%)

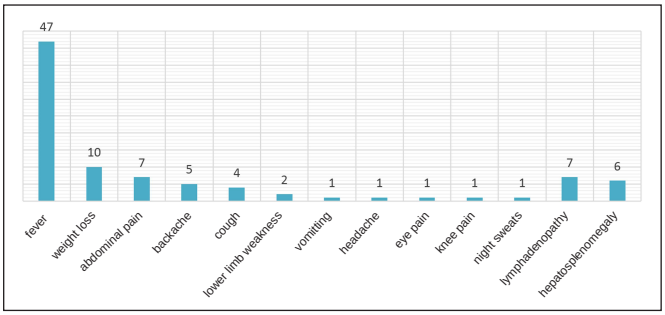

Fig 5. Signs and symptoms in patients

in blood culture, but the yield is low (40-70%)[9]. In our study blood cultures were performed in 83% of patients and those held for two weeks came out positive (11.3%). However, cultures from other sites like bone marrow have higher sensitivity, and better yield especially in patients taking antibiotics. Brucella species can also be cultured from pus, tissue, cerebrospinal fluid (CSF), and pleural / joint / ascitic fluid.[10-12]

Brucellosis is a zoonotic disease and history of contact with livestock and cattle helps in narrowing down the diagnosis. In our study 15% of patients gave contact history with animals or livestock. 81% of patients were not asked about any contact history. This emphasizes the importance of taking history and making differentials in patients with fever.

Diagnosis of brucellosis is made most by agglutination tests including Rose Bengal test, serum agglutination test, antiglobulin or Coombs' test, complement fixation, immunocapture test. Clinical correlation with titers above 1:160 by Standard Tube Agglutination Test (SAT) points towards high disease probability. However, in endemic areas, the titer of 1: 320 is taken as the cut-off. PCR-based tests are beneficial in relapsing and recurrent infections. Nowadays, these are also used to assess treatment efficacy.[14] Post-treatment follow-up to assess for relapse by PCR based assay was investigated by Morta and colleagues.[15]

In a study by Sascha al Dahouk[15] in Germany, a major symptom was fever (88%), which was significantly associated with hospitalization of the patient (OR 4.1; p<0.01). A total of (49%) patients reported fatigue; 43% reported arthralgia, 41% reported headaches, 27% reported massive sweating, 12% reported the loss of appetite, and 13% reported the loss of weight.[15] In our study, major symptom reported in patients (n=47) was fever, followed by weight loss (n=10). The least common symptoms were vomiting, headache, night sweats, and joint aches.

The occurrence of brucellosis is low in developed countries due to access and availability of animal screening programs.[16] In our study, the major incidence of brucella was reported in year 2016 followed by a second spike in the year 2018. There is no system developed here in Pakistan for the screening of zoonotic diseases including brucellosis. Worldwide diagnosis is mainly done by isolating the organism and serum agglutination as well as ELISA-based serological tests.[17, 18]

A case-control study conducted in Saudi Arabia showed the association of brucellosis with consumption of unpasteurized dairy products derived from sheep and goats as well as with animal parturition.[19] Brucellosis was shown to be associated with occupations including being a farmer, shepherd, or microbiologist, and with drinking fresh milk and buttermilk in a study done in Yemen. Socio-economic and educational factors were also independent risk factors.[20] In Iran a study done showed that consumption of unpasteurized dairy products and contact with a positive brucella patient had high association with brucellosis.[21]

Diverse methodologies are available for diagnosing brucellosis. Different modalities of testing were used by Sascha al Dahouk, where 67% patients were diagnosed by serologic tests, 69% by serum agglutination test (SAT), 2% by complement fixation (CFT), and 9% by ELISA; in 19%, a positive SAT was confirmed by CFT or ELISA.[15] Serological tests were performed on 105 patients in our study and raised titers for specific species pointed towards the diagnosis with 64.7% patients having titers of >1:80.

A study from Pakistan in 2014 also showed similar results with highest seropositivity for both *B. abortus* and *B. melitensis* (78.40%), followed by *B. melitensis* (14.37%) and *B. abortus* (13.17%).[22] The study presented that the disease prevalence rate in different occupational groups was between 27.04% - 32.90%.[22] A study from Bangladesh reported seroprevalence of brucellosis in patients with prolonged fever of 2%.[23] Hospital-based study from Kenya indicated a prevalence rate of 13.7% in febrile patients.[24] A study from Saudi Arabia reported a frequency of 26.92% among patients who presented with fever and nonspecific symptoms.[25] In Iran, the prevalence of brucellosis has been reported between 0.5% – 10.9%.[4] Studies from Bangladesh and Kenya reported *B. abortus* seropositivity only in their subjects.[13,14] *B. melitensis* seropositivity was found in all subjects in a study conducted in Iran.[26] Hussein Ageely et al from Saudi Arabia reported that *B. melitensis* is the main cause of human brucellosis followed by *B. abortus*.[27] In our study 50.5% of patients had *B. melitensis* followed by *B. abortus in* 49.5% of patients.

Conclusion

Brucellosis is no doubt an important zoonotic infection. It is prevalent in livestock and probably quite prevalent in humans as well especially in developing countries like Pakistan.

This study focuses on establishing the importance of animal contact history in patients with pyrexia of unknown origin. The clinical, radiological, and laboratory findings in brucellosis play pivotal roles in establishing the diagnosis. It is also imperative to consider brucellosis as a differential diagnosis of chronic illnesses in humans having positive contact history with livestock despite the absence of characteristic disease features. Treatment is possible and the overall prognosis is good.

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Conflict of interest
All authors have no conflict of interest

Contribution to authorship
BJ: conceptualization and supervision; AA: methodology, data curation, and analysis; MI: data curation, writing-original draft, and editing. All authors read and approved the final manuscript.

References

1. Ali Zamani , Soheil Kooraki , MD , Razieh Adabi Mohazab, *et al*: Epidemiological and clinical features of Brucella arthritis in 24 children. *Trop Doct* 2005, 35:229-31. 10.4103/0256-4947.81543
2. Minas M, Minas A, Gourgulianis K, Stournara A: Epidemiological and clinical aspects of human brucellosis in Central Greece. *Jpn J Infect Dis* 2007, 60:362-6.
3. Gwida M, Al DS, Melzer F, *et al*.: Brucellosis - regionally emerging zoonotic disease? *Croat Med J* 2010, 51:289-95.10.3325%2Fcmj.2010.51.289
4. Sofian M, Aghakhani A, Velayati AA, *et al*.: Risk factors for human brucellosis in Iran: a case-control study. *Int J Infect Dis* 2008, 12:157-61. 10.1016/j.ijid.2007.04.019. Epub 2007 Aug 14.
5. Corbel M : Brucellosis in Humans and Animals: FAO, OIE, WHO. Available. 2006.
6. Roth F, Zinsstag J, Orkhon D, *et al*.: Human health benefits from livestock vaccination for brucellosis: case study. 2003, 81:867-876.
7. Madkour MM, Madkoure: Brucellosis. New York. Springer Verlag; 2001.
8. Alkahtani, A.M., Assiry, M.M., Chandramoorthy, H.C. *et al*. Sero-prevalence and risk factors of brucellosis among suspected febrile patients attending a referral hospital in southern Saudi Arabia (2014–2018). *BMC Infect Dis* 20, 26 (2020). https://doi.org/10.1186/s12879-020-4763-z
9. Pablo Yagupsky: Blood Cultures for the Diagnosis of Human Brucellosis. In: Manal Mohammad Baddour (Eds.), Updates on Brucellosis - November 2015. https://doi.org/10.5772/61143
10. Colmenero JD, Reguera JM, Cabrera FP: Serology, clinical manifestations and treatment of brucellosis. *Infection*1990, 18:152-56. 10.1016/S1995-7645(11)60111-9
11. Memish Z, Mah MW, Al Mahmoud S, Al Shaalan M, Khan MY: Brucella bacteremia: clinical and laboratory observations in 160 patients. *J Infect Dis* 2000, 40:59-63.
12. Mantur BG, Biradar MS, Bidri C, *et al*.: Protean clinical manifestation & diagnostic challenges of human brucellosis in adults: 16 yrs experience in an endemic area. *J Med Microbiol* 2006, 55:897-903. 10.1099/jmm.0.46097-0
13. Nimri LF: Diagnosis of recent and relapsed cases of human brucellosis by PCR assay. *BMC Infect Dis* 2003, 3:5.
14. Morta P, Queiyo -ortuno ML, Rodrignej TA, Ordina A: Specificity of a polymerase chain reaction assay of a target sequence on the 31 Kilodalton Brucella antigen DNA used to diagnose human Brucellosis. *Eur J Clin Microbiol Infect Dis* 2001, 20:27-31.
15. Al Dahouk S, Neubauer H, Hensel A, *et al*.: Changing epidemiology of human brucellosis, Germany, 1962-2005. 10.3201%2Feid1312.070527
16. Lopes LB, Nicolino R, Haddad JPA: Brucellosis - risk factors and prevalence: a review. *Open Vet Sci J* 2010, 4:72-84. http://dx.doi.org/10.2174/1874318801004010072
17. Sadrizadeh B: Communicable disease control programmes in the Eastern Mediterranean Region of the World Health Organization. *Archives of Iranian medicine* 1999, 2:28-37.
18. Corbell JM: Brucellosis: an overview. *Emerg Infect Dis* 1997, 2:213-21. 10.3201%2Feid0302.970219
19. Cooper CW: Risk factors in transmission of brucellosis from animals to humans in Saudi Arabia. Department of Community Medicine, University of Sydney. Croydon, NSW, Australia; 10.1016/0035-9203(92)90575-W
20. Al-Shamahy HA, Whitty CJ, Wright SG: Risk factors for human brucellosis in Yemen: a case control study. Faculty of Medicine & Health Sciences, University of Sana'a Yemen. 10.1017/S0950268899004458
21. Sofian M, Aghakhani A, Velayati AA, Banifazl M, Eslamifar A, Ramezani A: Risk factors for human brucellosis in Iran: a case-control study. Arak Medical University Arak, Iran. 10.1016/j.ijid.2007.04.019
22. Shahid M, Basit A, Khan MA: Prevalence of Brucellosis among the Hospital Patients of Peshawar. Khyber Pakhtunkhawa. *J Infect Mol Biol* 2014, 2:19-21. 10.14737/jimb.2307-5465/2.2.19.21
23. Rehman AA, Berkvens D, Saegerman C, *et al*.: Seroprevalence of brucellosis in patients with prolonged fever in Bangladesh. *J Infect Dev Ctries* 2016, 10:939-46. 10.3855/jidc.6844
24. Njeru J, Melzer F, Wareth G, *et al*.: Human Brucellosis in Febrile Patients Seeking Treatment at Remote Hospitals, Northeastern Kenya, 2014-2015. *Emerg Infect Dis* 2016, 22:64. 10.3201%2Feid2212.160285
25. Alrheam AI, Al Shehri ZA, Elneam AI, Cruz CP: Human Brucellosis Incidence Trends in Central Saudi Arabia (DawadmiGovernate). *Int J Adv Res* 2015, 3:1580-6.
26. Wojno JM, Moodley C, Pienaar J, *et al*.: Human Brucellosis in South Africa: Public health and diagnostic pitfalls. *S Afr Med J* 2016, 106:835-5. 10.7196/samj.2016.v106i9.11020
27. Ageely H, BanSi I, Gaffar A, *et al*.: Prevalence and Risk Factors for Brucellosis in Jazan Province. Saudi Arabia. *Trop J Pharm Res* 2016, 15:189-94. 10.4314/tjpr.v15i1.26