National Journal of Biological Sciences

Received: 10th June, 2024
Revised: 21st October, 2024
Accepted: 22nd November, 2024
Published: 26th December, 2024

DOI: https://doi.org/10.37605/v5i2/2

RESEARCH PAPER

TITLE:

PREVALENCE OF *BRUCELLOSIS* AMONG FEBRILE PATIENTS IN RAWALPINDI / ISLAMABAD AREA

Authors: Sidra Jabeen[1], Ayesha Kashif[2], Syeda Kainat Ali[3], Mustaqeem Shahzadi[4], Ali Hussnain[5], Muhammad Fahad Anwar[6], Sudais Hashmi[7], Sadaf Akhlaq[8], Dua Zhaira[*]

AFFILIATIONS

[1]Departmentof Allied Health Sciences, Superior University, Sargodha

[2]Department of Medicine and Biochemistry, Bahria University Health Sciences Campus, Islamabad

[3,4] The superior University Lahore Sargodha campus

[*]Faculty of Rehabilitation and Allied Health Sciences, Riphah International University, Islamabad, Pakistan

[5,8]Abasyn University, Islamabad

[6,7] Riphah International University, Rawalpindi

Corresponding author: *Duazhaira@gmail.com*

PREVALENCE OF *BRUCELLOSIS* AMONG FEBRILE PATIENTS IN RAWALPINDI / ISLAMABAD AREA

ABSTRACT

Human *brucellosis*, an important zoonosis is a chronic occupational disease that mostly affects butchers, slaughter-house workers, animal keepers, veterinarians and laboratory workers. The causative agent is transmitted through direct contact with infected tissues or fluids by infected animals, ingesting unpasteurized milk and meat products, and inhalation of infectious aerosols. It can be transmitted vertically or horizontally due to close contacts like sexual intercourse (especially in animals but rare in humans). In humans, the disease generally, begins as an acute febrile illness with non-specific flu-like signs and then leads to intermittent fever with manifestations such as cardiovascular, genitourinary, haematopoietic, nervous, skeletal, pulmonary, gastrointestinal, ocular, and cutaneous involvement

The objective of the study is to determine the frequency of serologically positive *brucellosis* among febrile patients at Rawalpindi / Islamabad area.

Hospital based, observational, case control study in patients of *Brucellosis*.

The Department of Microbiology at Armed Forces Institute of Pathology, Rawalpindi Pakistan from October 2020 to January 2021. Direct agglutination test and Enzyme-linked Immunosorbent assay, both tests were used for serology of *brucellosis*.

Out of 207 patients, 19 patients were found positive for *Brucellosis* whereas 188 were negative. It has also been reported that out of 19 positive patients, 11 were males while 8 were females. We found that the majority of patients were found negative for *Brucellosis*. The frequency of *Brucellosis* among febrile patients was high. In conclusion we postulated that the frequency of *Brucellosis* among febrile patients is 9.2%. Out of these positive cases, 58% patients were male while on the other hand 42% were female, showing male predominance.

KEYWORDS: Brucellosis, Febrile Patients, Musculoskeletal Pain, Undulating Fever

1. INTRODUCTION

Brucellosis is a zoonotic disease caused by the *Brucella* bacteria, which can infect both animals and humans. The disease is characterized by a range of nonspecific symptoms, including fever, fatigue, and musculoskeletal pain, making it challenging to diagnose. (Köse et al., 2014) *Brucellosis* is particularly prevalent in resource-limited regions, such as the Middle East, where it remains a significant public health concern. (Heydari, 2019) The Rawalpindi and Islamabad areas of Pakistan are endemic for brucellosis, with a high disease incidence among the local population. The region's proximity to areas with a high prevalence of *Brucella*-infected livestock, as well as the local population's reliance on animal-based products, contribute to the ongoing challenge of managing this disease (Shehzad et al., 2020).

In humans, the disease generally, begins as an acute febrile illness with non-specific flu-like signs and then leads to intermittent fever with manifestations such as cardiovascular, genitourinary, haematopoietic, nervous, skeletal, pulmonary, gastrointestinal, ocular, and cutaneous involvement (Aygen, B., et al.,2002). Chronic cases of *brucella* are due to their ability to survive and multiply in macrophages. In human *Brucella meletensis* infections tend to be more severe and prolonged. Various names used for human brucellosis are Malta fever, Undulant fever and Mediterranean fever. The principle manifestations of animal brucellosis are reproductive failure, i.e., orchitis, and epididymitis in males, and abortion / birth of unthrifty offspring in female (Rahman, M., et al.,2006) *B.abortus* is the most frequent causative agent in bovines. It has been eradicated from Canada, Japan, Australia, Various northern and central European countries, New Zealand, and from farmed cattle in the U.S.A(Ali, S., et al.,2017). It remains a major public health problem in Middle east, Mediterranean region, Latin America, Africa and parts of Asia.

The low index of suspicion, under diagnosis or misdiagnosis, and lack of sufficient knowledge about the disease have been attributed to the wide spread of the disease (Thakur, S., et al.,2002). Generally, it is considered that *brucellosis* is under diagnosed in many areas where it is endemic. In developing as well as malaria and typhoid endemic countries, after the exclusion of malaria and typhoid, fever is often managed solely on clinical symptoms using non-specific antibiotic regiments that do not cover *Brucella* species (Migisha, R., et al., 2018). If left untreated the disease can progress to chronic illness.

Pakistan is an agricultural country with livestock being most common in villages all over the country. Although *brucellosis* is endemic in Pakistan, the exact frequency of *brucellosis* in different parts of our country is not well known resulting in under-diagnosis/misdiagnosis of the disease. To address this issue, it is crucial to investigate the frequency of brucellosis among febrile patients in the Rawalpindi and Islamabad areas, which will inform public health strategies and targeted interventions.

2. MATERIALS AND METHODS

The cross-sectional descriptive design is used in our study. The study carried out at the department of Microbiology, Armed forces institute of pathology, Rawalpindi. The study participants are the patients of all ages and gender with febrile illness and clinical suspicion of brucellosis. Data is collected in 4 months from October 2023 to January 2024 after approval from institutional ethical committee. A total of 207 blood samples in serum gel tubes are processed during the study period. The sample size is collected by using a seroprevalence of 16% (Ali, Nawaz et al. 2018) with the help of WHO sample size calculator .The nonprobability convenience sampling technique was used to collect data.

2.1 INCLUSION CRITERIA:

The blood samples of patients of all ages and gender with acute febrile illness were included.

2.2 EXCLUSION CRITERIA:

- Improperly collected samples
- Repeated samples from the same patient.

2.3 DATA ANALYSIS:

Statistical analysis of the data was performed using IBM **SPSS** statistics Version. 23.00. Qualitative data was analyzed for frequencies and percentages. Quantitative data was analyzed for mean and standard deviation (**SD**). Microsoft Excel 2016 was used for making tables, graphs and calculations.

2. 4 ETHICAL CONSIDERATIONS:

Approval from the institutional ethical committee, Armed Forces Institute of Pathology (AFIP) Rawalpindi was taken and following ethical considerations were also applied:

- Informed consent was taken from the patients.
- Data was kept confidential.
- Data was used for academic purpose only.

2.5 SAMPLE COLLECTION

3-5ml blood sample each of the febrile patients with suspected brucellosis was collected in yellow or red topped serum tubes. Then these blood samples were centrifuged at 4000rpm for 2 minutes in order to obtained serum.

2.6 SEROLOGY

Direct agglutination test **(DAT**) using *Brucella abortus* and *Brucella melitensis* antisera (Atlas Medical) and Brucella IgM antibodies using Enzyme-Linked Immunosorbent Assay **(ELISA**) techniques (CALBIOTECH) were performed on each serum sample.

2.7 DIRECT AGGLUTINATION TEST:

In a qualitative direct agglutination test, 50**µl** of the patient serum was mixed with the one drop (50**µl)** of *Brucella abortus* and *Brucella melitensis* anti sera in a separate circle of disposable reaction card; visible agglutination appeared if serum contains IgG or IgM antibodies against Brucella species. A quantitative test was done if DAT is positive. In quantitative test serial dilutions (1/20,1/40,1/80,1/160,1/320) of serum were tested as per manufacturer guidelines, the highest dilution of the serum giving positive result was the titre i.e. 1/20,1/40,1/80,1/160,1/320 respectively. A four-fold rise in *Brucella* agglutination titer in paired sera or Titer **≥1/160** for a single specimen was taken as positive.

2.8 ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA):

In **ELISA** diluted patient serum was added to the wells coated with purified antigen. If IgM specific antibody is present in the serum it would bind to the antigen. Washing was done to remove unbound antibodies and the enzyme conjugate was added to bind to the antigen-antibody complex if present. Excess enzyme conjugate was washed off and then the substrate was added. The plate was incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated would be proportional to the amount of IgM specific antibody in the sample. IgM antibodies result **>1.1** was considered as positive as per kit manufacturers guidelines.

3. RESULTS

A total of 207 samples were recruited to observe frequency of *brucellosis*. Both Direct agglutination test and Enzyme linked

immunoassay were performed on each sample of febrile patient. Out of these 207 samples 19 (9.2%) were positive for brucellosis and 188 (90.8%) were found negative by both DAT and ELISA. Descriptive statistics (Mean ± SD) for continuous variable like age was measured. Out of 19 positive samples 11 were males (58%) and 8 (42%) were females

The mean age was 38.5± 17.9 standard deviation calculated through statistical analysis (Statistical package for social science; SPSS version 23).

4. DISCUSSION

Human *brucellosis*, an important zoonosis is a chronic occupational disease that mostly affects butchers, slaughter-house workers, animal keepers,veterinarians and laboratory workers.The causative agent is transmitted through direct contact with infected tissues or fluids by infected animals, ingesting unpasteurized milk and meat products, and inhalation of infectious aerosols. It can be transmitted vertically or horizontally due to close contacts like sexual intercourse (especially in animals but rare in humans). In humans, the disease generally, begins as an acute febrile illness with non-specific flu-like signs and then leads to intermittent fever with manifestations such as cardiovascular, genitourinary, haematopoietic, nervous, skeletal, pulmonary, gastrointestinal, ocular, and cutaneous involvement.

Brucellosis was first discovered in 1850s in Malta (Wyatt 2013). In 1886, David Bruce isolated the causative organism from four fatal cases of Malta fever and named it *Micrococcus melitensis*(Bruce,D.,1887). In 1897 Bernard Bang, a physician-veterinarian, isolated an organism named bacillus abortus while studying a disease of cattle "contagious abortion". In 1914 similar organism was also discovered from swine.

In 2020 a cross-sectional sero-epidemiological study was conducted by Alkahtani, A. M., et al in Aseer Central Hospital, South Saudi Arabia. Out of 7567 cases the highest prevalence rate was observed in age group 21–40 year old (40.5%) followed by 41–60 years (27.7%). The lowest prevalence rate was noticed in old and young children (15 and 3%, respectively). (Alkahtani, A. M., et al.,2020)

A perspective study titled "prevalence and risk factors of Brucellosis among febrile patients attending a community hospital in South western Uganda" was conducted by Migisha, R., et al. in 2018. According to it a total of 235 patients were enrolled, prevalence of brucellosis was 14.9%, with a culture confirmation in 4.3% of the participants.(Migisha, R., et al. 2018)

The present study was conducted to detect the frequency of *Brucellosis* among febrile patients. An extensive amount of studies were reported internationally on the seroprevalence of Brucellosis. It was noted that the frequency of Brucellosis in our patients from Pakistan were quite different with that reported in the previous international studies (40.5% and 14.9%). Out of 207 samples, only19 were found positive while 188 were negative for this mutation.

This study was conducted to highlight the frequency of Brucellosis in febrile patients and their association with gender. In our study out of 19 positive cases,11(58%) patients were male while on the other hand 8(42%) were female.

5. CONCLUSION

The purpose of the study is to explore the frequency of brucellosis among febrile patients and to find the proportion of febrile patients serologically positive to have brucellosis thereby helping physicians to keep brucellosis in the differential diagnosis and timely management of the patients. This study showed that the frequency of Brucellosis among febrile patients is 9.2%. Out of these positive cases, 58% patients were male while on the other hand 42% were female. In conclusion; we postulated that the epidemiological significance of Brucellosis among febrile patients is very high in Pakistani population with male predominance.

REFRENCES

1. Ahmadu, B., Sikazwe, M.S., Sakala, R. and Pandey, G.S., 1999. Sero-prevalence of bovine brucellosis in cattle at Lusaka abattoirs. Bulletin of Animal Health and Production in Africa, 47, 119–121

2. Al-Shamahy, H.A., Whitty, C.J.M. and Wright, S.G., 2000. Riskfactors for human brucellosis in Yemen: a case control study. Epidemiology and Infection, 125, 309–313

3. Alton, G.G., Jones, L.M. and Pietz, D., 1975. Laboratory Tech niques in Brucellosis (World Health Organization, Geneva), 63–34

4. Anon., 2000. Annual Report of the Department of Research and Specialist Services, (Government Printers, Lusaka Zambia), 1–18

5. Bell, L.M., Hayles, L.B. and Chanda, A.B., 1976. Evidence of reservior hosts of Brucella melitensis. Medical Journal of Zambia, 10, 152–153

6. Bell, L.M., Hayles, L.B. and Chanda, A.B., 1977. Serological evidence of Brucella melitensis infection in goats and eland in Zambia. Veterinary Record, 101, 305

7. Dooho, I., Martin, W. and Stryhn, H., 2003. Veterinary Epidemi ologic Research, (AVC Inc., Charlottetown), 35–42

8. England, T., Kelly, L., Jones, R.D., MacMillan, A. andm Wooldridge, M., 2004. A simulation model of brucellosism spread in British cattle under several testing regimes. Pre ventive Veterinary Medicine, 63, 63–73

9. Ghirotti, M., Semproni, G., De Meneghi, D., Mungaba, F.N., Nannini, D., Calzetta, G. and Paganico, G., 1991. Sero prevalences of selected cattle diseases in the Kafue flats of Zambia. Veterinary Research and Communications, 15,25–36

10. Godfroid, J., Michel, P., Uytterhaegen, L., Desmedt, C., Rasseneur, F., Boelaert, F., Saegerman, C. and Patigny, X., 1994.

11. Brucellose enzootique a Brucella suis biotype 2 chez le sanglier (Sus Scrofa) en Belgique [Brucella-suis Biotype 2 Infection of wild boars (Sus Scrofa) in Belgium]. An naesde Medecine Veterinaire ´ , 138, 263–268

12. Godfroid, J., Saegerman, C., Wellemans, V., Walravens, K., Letesson, J.J., Tibor, A., McMillan, A., Spencer, S., Sanna, M., Bakker, D., Pouillot, R. and Garin-Bastuji, B., 2002.

13. How to substantiate eradication of bovine brucellosis when aspecific serological reactions occur in the course of brucellosis testing. Veterinary Microbiology, 90, 461–477

14. Jiwa, S.F.H., Kazwala, R.R., Tungaraza, R., Kimera, S.I. and Kalaye, W.J., 1996. Bovine brucellosis serum agglutination test prevalence and breed disposition according

to prevalent management systems in the Lake Victoria zone of Tanzania.
15. Preventive Veterinary Medicine, 26, 341–346 Jordan, D., 1995. Herdacc: a program for calculating herd level (aggregate) sensitivity and specificity (Guelph, ON, Canada, N1G2W1, Department of population medicine, University of Guelph)