Prevalence of Human Brucellosis in a Suspected Population of District Swat

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ABSTRACT

Background: Brucellosis is caused by a gram-negative, aerobic, non-spore forming, coccobacillus, transmitted by consumption of contaminated dairy products. Close contact with infected animals is also a risk factor. Diagnosis is difficult due to non-specific manifestations like fever, back pain, arthralgia, muscle aches, and anemia, thereby diagnosis is based on laboratory tests. Although under rated, 50,000 new cases of human brucellosis worldwide are reported annually. In Pakistan, despite the disease being endemic, data is limited. Moreover, the increased consumption of raw dairy products has posed an additional risk.

Objective: To identify the prevalence of human brucellosis in the suspected population of District Swat.

Material and Methods: This cross-sectional study was conducted at a tertiary-care hospital, of Swat from November 2017-October 2018. From the outpatient department, 150 participants were enrolled being suspected of having human brucellosis. Every participant was screened for Brucella antibodies by Serum Plate Agglutination Test (SPAT) and confirmed through Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR).

Results: The mean age of the participants was 29.65±11.27 years, 34% of which were 21-30 years of age. Most (62%, n=93) were females and a greater proportion of them consume fresh milk (45.3%), followed by fresh milk and milk pack both (30%). Around 119 (79.3%) were positive on SPAT, 50.7% on ELISA, and 49.3% on PCR with a significantly higher proportion in females (p=0.001).

Conclusion: Human brucellosis was found to be highly prevalent among suspected population exposed to raw dairy products with a definitive diagnosis found in almost half of the samples collected.

Key Word: Human brucellosis, livestock, SPAT, ELISA, PCR, zoonotic.

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INTRODUCTION

Globally, brucellosis is one of the widespread zoonotic infection being caused by the bacterial species-genus *Brucella* a gram-negative aerobic, non-spore forming coccobacilli.¹⁻² The disease is mainly transmitted among humans through consumption of the dairy product that is contaminated.³⁻⁵ However, close contact with infected animals i.e. meat inspectors, veterinarians, animal health technicians, abattoir workers and laboratory workers working in microbiology labs are also at risk of brucellosis transmission, thereby Centre of Disease Control (CDC) has declared brucellosis an occupational disease.⁴⁻⁵ World Health Organisation (WHO) has identified brucellosis as one of the most significant but highly neglected zoonotic disease globally.⁶⁻⁷ Brucellosis has been responsible for the significant economic impact as it causes decreased milk production, abortion, and infertility in goats, sheep, swine and camel.⁸⁻¹⁰

The most virulent brucella species for the human is *Brucella melitenensis*, although *B. abortus*, *B. canis*, marine brucella strains (i.e. *B. ceti* and *B.*

pinnipedialis) and *B. inopinata* can lead to serious infections.¹¹⁻¹² Clinical signs and symptoms of human brucellosis included undulating fever, back pain, arthralgia, increased sweating, abdominal pain, muscle aches, headache and change in personality with psychiatric illness.¹³⁻¹⁴ Moreover, other symptoms being associated with human brucellosis have been leucopenia, anemia, thrombocytopenia, arthritis, meningitis and endocarditis.¹⁴ The brucellosis is difficult to diagnose on the basis of clinical signs and symptoms due to non-specific manifestations; thereby diagnosis is based on laboratory tests.¹⁴ The brucella enters the body through the digestive tract, lungs, and intact skin, from where it spread through blood and lymphatic system to other organs, infecting the tissue and causing localized infection.¹⁵

Globally, the precise incidence of human brucellosis is not known, however, approximately 50,000 new cases of human brucellosis are reported annually. WHO has under rated this figure, and announced that the true incidence of human brucellosis is ten times the figure officially announced.² Moreover, in Pakistan the disease is endemic but few studies being conducted and data being limited.¹⁶ Moreover, the increased consumption of raw dairy products from cows, sheep, goats, and buffalos have posed an additional risk of brucellosis transmission.¹⁷

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Thermal treatment i.e. heating or boiling milk to a minimum temperature of 80 to 90 °C for several minutes can be used to control brucellosis.¹⁸ At the farm level, Rose Bengal Test (RBT), a simple, rapid and cost-effective test appeared to be effective. Moreover, in some countries, the vaccination campaign has proven effective.¹⁹

Considering, there is limited data available related to the prevalence of human brucellosis, though a rise in consumption of raw dairy products in Pakistan, the study was conducted to identify the prevalence of human brucellosis suspected population of District Swat.

MATERIAL AND METHODS

This descriptive cross-sectional study was conducted in the Department of Medicine Saidu teaching hospital, Swat district, a tertiary care hospital, for a period of one year from November 2017 to October 2018. One hundred and fifty participants were enrolled in the assigned duration and following the inclusion criteria through non-probability consecutive sampling techniques from the outpatient clinic of the Department of Medicine, Saidu teaching hospital being suspected for having signs and symptoms of human brucellosis. Patients of either gender with age greater than 18 years visiting to seek medical help with clinical signs and symptoms for human brucellosis (i.e. weakness, lethargy, periodic fever, sweating, decreased appetite, arthralgia, myalgia, weight loss, back pain, and headache) were selected by the clinician to be recruited in this study. Patients with a confirmed diagnosis of tuberculosis or having a history of being suffered from tuberculosis previously were not considered for recruitment in this study. The patients not willing to give informed consent, unable to understand the study procedure due to psychiatric illness and pregnant women were excluded.

From each participant, 5 ml of blood was drawn using an aseptic technique. Each sample was centrifuged for 5 minute and serum separated was stored at -20 °C for further analysis. Initially, the sample was screened through Serum Plate Agglutination Test (SPAT) for Brucella antibodies for the diagnosis of brucellosis. Samples showing positive results with SPAT were further processed through Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) as per the recommended guidelines and standard procedures. Apart from the confirmation of the

diagnosis of human brucellosis, the data on socio-demographic conditions (i.e. age in years, gender, education, ethnicity, occupation, and income) was collected through closed-ended questions at enrollment. Moreover, the study participants were also inquired about milk consumed and livestock from which milk was obtained.

The present study was conducted according to the ethical guidelines of the Helsinki declaration and Pakistan Medical and Research Council (PMRC) and the study was initiated after the approval from the Ethical Review Committee (ERC). Written informed consent was obtained from all the participants prior to recruitment, where they were comprehensively explained about the study objective, research processes involved, risks and benefits associated with the research. Importantly, the study participant's confidentiality and anonymity were maintained throughout the research process.

Data Analysis

The data collected were analyzed using SPSS version 21 (IOBM). The data recorded on the predesigned structured proforma was entered in the SPSS software and validated twice for any incorrect entries. Descriptive statistics were performed. The categorical variables were reported as frequency/ percentage; while the quantitative variables were reported as Mean $\pm$ Standard Deviation. The chi-square statistics were used to identify the association between the presence of human brucellosis (positive diagnosis) with age categories and gender. If the assumptions of chi-square were not satisfied, Fisher exact test was used. The p-value of less than 0.05 was considered significant.

RESULTS

Table 1 gives details of the socio-demographic characteristics of one hundred and fifty study participants who were recruited in the study. The mean (SD) age of the study participant was 29.65 (11.27) years, with majority (34%) having age 21 to 30 years, followed by age, less than or equal to 20 years (25.3%), age lying between 31 to 40 years (24.7%) and age greater than 40 years (16%). The majority, ninety-three (62%) of the study participants were females. The education level of the study participant was fairly distributed. The majority, around thirty-eight percent, had an education of five years or less, around twenty-eight percent had attained education of six to ten

years, nearly fifteen percent were intermediate and around nineteen percent had an education level of bachelors and above. Moreover, the majority was a housewife (44.7%), followed by self-employed (19.3%), student (15.3%), professional (10%), laborer (8.7%), professional (10%) and retired not working (2%). The most pre-

dominant ethnicity was Pashtun (78.7%), followed by Punjabi (9.3%). There were seventy-seven study participants (51.3%) having income (less than 15,000 PKR), followed by around thirty-five percent having income (15,000 to 50,000 PKR), while only twenty participants (13.3%) had an income of 50,000 Pakistani rupees or more.

Table 1. Socio-demographic characteristics of the study participants

Socio-demographic characteristics	n (%) OR Mean $\pm$ SD (N = 150)
Age (years)	29.65 $\pm$ 11.27
Age categories (years)	
Less than or equal to 20 years	38 (25.3)
21 - 30	51 (34)
31 - 40	37 (24.7)
41 - 50	19 (12.7)
Greater than 50 years	5 (3.3)
Gender	
Male	57 (38)
Female	93 (62)
Education	
Five years or less	57 (38)
6 to 10 years	41 (27.3)
Intermediate	23 (15.3)
Bachelors	18 (12)
Masters	11 (7.3)
Occupation	
Housewife	67 (44.7)
Student	23 (15.3)
Laborer	13 (8.7)
Self-employed	29 (19.3)
Professional	15 (10)
Retired not working	3 (2)
Ethnicity	
Punjabi	14 (9.3)
Urdu Speaking	3 (2)
Sindhi	4 (2.7)
Pashtun	118 (78.7)
Balochi	11 (7.3)
Income	
Less than PKR 15,000	77 (51.3)
PKR 15,000 to 50,000	53 (35.3)
Greater than PKR 50,000	20 (13.3)

Table 2. Milk consumed and livestock from which milk obtained by the study participants

Milk consumed and livestock details	n (%) (N = 150)
Milk Consumed	
Fresh	68 (45.3)
Milk Pack	37 (24.7)
Mix (Fresh and Milk Pack both)	45 (30)
Livestock	
Cow	33 (22)
Buffalo	51 (34)
Cow and Buffalo	8 (5.3)
Goat	4 (2.7)
Nil	54 (36)

Table 2 gives details of milk consumed and the livestock from which the milk was obtained by the study participants being recruited in this study. Majority of the study participants consume fresh milk (45.3%), followed by fresh milk and milk pack both (30%) and only around one quarter consume milk pack. The livestock from which the milk was obtained were as follows; cow (22%), buffalo (34%), cow and buffalo (5.3%) and goat (2.7%).

Figure 1 gives details of the prevalence of human brucellosis diagnosed positive through SPAT, ELISA, and PCR. Among one hundred and fifty participants enrolled, one hundred and nineteen (79.3%) were diagnosed for human brucellosis on SPAT. However, the confirmation of diagnosis for human brucellosis on ELISA was (50.7%). Moreover, there were seventy-four participants (49.3%) being confirmed positive for human brucellosis on PCR (49.30%).

Figure 1. Prevalence of human brucellosis diagnosed positive through SPAT, ELISA, and PCR

Table 3 entails details about the prevalence of human brucellosis diagnosed positive through ELISA and PCR across age categories and gender. There was no significant difference in the positive diagnosis of human brucellosis through ELISA across age categories. The distribution of positive diagnosis of human brucellosis through ELISA across age categories were; less than or equal to 20 years (31.6%), 21 to 30 years (34.2%), 31 to 40 years (19.7%), 41 to 50 years (10.5%) and greater than 50 years (3.9%). Moreover, a significant difference was observed in the positive diagnosis of human brucellosis through ELISA across gender. A greater proportion of females had a positive diagnosis of human brucellosis through ELISA (90.8% vs. 32.4%; p-value = 0.001). Furthermore, no significant difference was observed in the positive diagnosis of human brucellosis through PCR across age categories. The distribution of positive diagnosis of human brucellosis through PCR across age categories were; less than or equal to 20 years (31.1%), 21 to 30 years (33.8%), 31 to 40 years (20.3%), 41 to 50 years (10.8%) and greater than 50 years (4.1%). Importantly, a significant difference was observed in the positive diagnosis of human brucellosis through PCR across gender. Greater proportion of females had positive diagnosis of human brucellosis through PCR (90.5% vs. 34.2%; p-value = 0.001).

Table 3. Prevalence of human brucellosis diagnosed positive through ELISA and PCR across age categories and gender (N = 150)

across age categories and gender (N = 150)			
Age categories and Gender	Positive n (%)	Negative n (%)	P-value
Diagnosis through ELISA			
Age categories (years)			
Less than or equal to 20 years	24 (31.6)	14 (18.9)	0.328
21 – 30 years	26 (34.2)	25 (33.8)	
31 – 40 years	15 (19.7)	22 (29.7)	
41 – 50 years	8 (10.5)	11 (14.9)	
Greater than 50 years	3 (3.9)	2 (2.7)	
Gender			
Male	7 (9.2)	50 (67.6)	0.001
Female	69 (90.8)	24 (32.4)	
Diagnosis through PCR			
Age categories (years)			
Less than or equal to 20 years	23 (31.1)	15 (19.7)	0.452
21 – 30 years	25 (33.8)	26 (34.2)	
31 – 40 years	15 (20.3)	22 (28.9)	
41 – 50 years	8 (10.8)	11 (14.5)	
Greater than 50 years	3 (4.1)	2 (2.6)	
Gender			
Male	7 (9.5)	50 (65.8)	0.001
Female	67 (90.5)	26 (34.2)	

The present study conducted had a number of limitations. Firstly, the study was conducted in only hospital setting. Secondly, suspected participants were enrolled through a non-probability consecutive sampling technique, which would have induced the selection bias. Moreover, limited only one hundred and fifty participants, were enrolled in this study. Importantly, the hospital-based study was conducted which might have not reflected the true prevalence of human brucellosis. Thus, a consecutive sampling technique, a limited number of study participants and recruitment from only study site had limited the generalisability of the study findings. In the future, a community-based study is desirable to be conducted with the recruitment of larger samples through random selection for a more precise estimate of the prevalence of human brucellosis.

CONCLUSION

Human brucellosis was found to be highly prevalent among suspected population exposed to raw dairy products with a definitive diagnosis found in almost half of the samples collected. This infection is evolving in Pakistan and requires monitoring, declaration, vaccination, and the establishment of a well-structured control and eradication program.

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