



Short Communication

Seroprevalence of Brucellosis in Occupationally Exposed Humans in Quetta, Pakistan

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Abstract | Brucellosis is a worldwide zoonosis. It is transmitted by direct contact or consumption of contaminated unpasteurized dairy milk. A total of 193 sera were collected from occupationally exposed participants in Quetta, Pakistan and analyzed by Rose Bengal Plate Test (RBPT). An overall 4/193 (2.1%, CI 0.6-5.2) seropositive were found. A higher seropositive rate was found in participants with >40 years age, livestock contact, poor socioeconomic group and no raw milk consumption. All variables didn't show statistical significance by Fischer's exact test. Further epidemiological studies and awareness programs are recommended. Based on the results and review of the literature, the authors urge to consider higher age ≥ 40 years, direct contact and raw milk intake as risks for the infection.

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Introduction

Brucellosis is a worldwide zoonotic disease caused by intracellular living Gram-negative bacteria of the genus *Brucella* (*B.*). In Pakistan, *B. abortus* is the main cause of brucellosis in bovines, *B. melitensis* in small ruminants and *B. canis* in dogs whereas *B. suis* has never been reported (Jamil *et al.*, 2021). Infected

animals shed the organism in body fluids or birth products e.g., vaginal fluid, milk or fetal membranes which act as source of direct and indirect transmission to humans and other animals. Additionally, consumption of contaminated raw/unpasteurized milk in humans remains a main source of infection (Hakeem and Saeed, 2019). The clinical picture is not pathognomonic but undulant fever, weakness,

splenomegaly with occasional reproductive problems are prominent symptoms (Qazilbash and Bari, 1997). Considered as one of the most frequent zoonosis in the world, it infects, an estimated 2.1 million humans worldwide every year (Laine et al., 2023). Brucellosis is one of the priority zoonotic diseases of the National Institute of Health (NIH) in Pakistan and calls have been made to tackle this disease under “One Health” paradigm (CDC, 2017; NIH, 2017). Sporadic cases in humans do occur every year throughout the country but are highly likely to be underreported (NIH, 2022). Balochistan is the largest province (44% by landmass) of the country but studies on human brucellosis are scarce (Ali et al., 2017; Jamil et al., 2021). In this scenario, the purpose of this study was to establish our understanding about the seroprevalence rate of occupationally exposed humans in the district Quetta of Balochistan, Pakistan (Figure 1).

Table 1: Univariable analysis of the risk factors of human brucellosis (n=193) in Quetta, Pakistan.

Variable	Category	Pos./ Tested	Prev. % (95% CI)
Age	> 40	2/36	5.6 (0.7-18.7)
	≤ 40	2/157	1.3 (0.2-4.5)
Occupation	Farm Worker	3/73	4.1 (0.9-11.5)
	Butcher	1/117	0.9 (0-4.7)
	Veterinarian	0/3	0 (0-70.8)
Contact with animals	Small ruminants	2/55	3.6 (0.4-12.5)
	Cattle	2/82	2.4 (0.3-8.5)
	Both	0/56	0 (0-6.4)
Previous infection	No	4/185	2.2 (0.6-5.4)
	Yes	0/8	0 (0-36.9)
Socioeconomic group	Poor	2/85	2.4 (0.3-8.2)
	Middle class	2/108	1.9 (0.2-6.5)
Raw milk intake	No	3/140	2.1 (0.4-6.1)
	Yes	1/53	1.9 (0-10.1)
Total		4/193	2.1 (0.6-5.2)

Materials and Methods

A total of 193 occupationally exposed persons (livestock farm workers, butchers and veterinarians) were recruited conveniently for this study. Biological plausible variables’ data (age, occupation, contact with animals, previous infection history, socioeconomic status and raw milk intake) were noted on a questionnaire proforma. Sera were separated and stored at -20 °C until further use. These sera were subjected to Rose Bengal Plate test (RBPT) using Pourquier®

Rose Bengal Antigen (IDEXX, Montpellier, France) along with positive and negative control sera provided by the Friedrich-Loeffler-Institut (FLI), Jena, Germany as described by World Organization for Animal Health (OIE, 2022). Briefly, 25μL of each serum were mixed in equal amounts of antigen on a white enamel plate at room temperature and waited for four minutes. A visible agglutination comparable to the positive control serum was considered as positive. Fischer’s exact test was performed on the variables by using SPSS Statistics (IBM Corporation, New York, USA).



Figure 1: Spatial presentation of the sampling site.

Results and Discussion

Four of 193 (2.1%, CI 0.6-5.2) sera were found positive by RBPT in the study. Age >40 years showed higher seroprevalence ratio (5.6%, CI 0.7-18.7) when compared to ≤40 years of age (1.3%, CI 0.2-4.5). Livestock farm workers showed higher seroprevalence (4.1%, CI 0.9-11.5). Seroprevalence (3.6%, CI 0.4-12.5) was found higher in the persons having contact with small ruminants. Poor socioeconomic status showed higher seroprevalence (2.4%, CI 0.3-8.2). Raw milk consumption showed a lower seroprevalence rate (1.9%, CI 0-10.1) than those who didn’t consume raw milk (2.1%, CI 0.4-6.1). A gender-based analysis could not be undertaken as all samples were from male subjects (Table 1). Similarly, multivariate analysis was not considered as all variables showed non-significant association with the seropositivity of the infection.

Although our study could not find a significant association with the factors, based on the previous published data, occupational exposure, duration and age should be considered as risks for brucellosis

transmission (Ali *et al.*, 2021). Previously, inadequate knowledge and at least one risky practice have been found in animal workers/owners in different areas of the country (Ali *et al.*, 2016; Arif *et al.*, 2017; Hussain *et al.*, 2021; Tahir *et al.*, 2022). Additionally, contact with the diseased animals and contaminated raw milk consumption could be associated factors in clinical human cases (Hakeem and Saeed, 2019; Saddique *et al.*, 2019). A more variable data for health status would be needed to confirm its association with the disease. Pasteurization of the milk before consumption is recommended as the bacteria is killed when boiled at 62-63 °C for 3 minutes (Murray *et al.*, 1932). Although brucellae respond well to various antimicrobials, culling of the infected animals remains a preferred choice to eliminate the infection. Awareness programs must be implemented.

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Novelty Statement

This study presents first detailed brucellosis seroprevalence among occupationally exposed individuals in Quetta, Balochistan. Despite being the largest province in Pakistan, prior research on human brucellosis in Balochistan are scarce. Our findings provide a foundation for future research and preventive measures in the region.

Author's Contribution

MS: Conceptualized and supervised this study.
MAK: Helped collecting the samples.
TJ, MHT and AR: Performed the laboratory work.
KKK and MHH: Performed the analysis.
TJ: Wrote the manuscript.
HN and MI: Reviewed the manuscript.

Ethical statement

The biological specimens were collected as per ethical and standard procedures of the Department of Clinical Medicine and Surgery, University of Agriculture,

Faisalabad 38000, Pakistan. All participants were prior informed about the possible outcomes of the study and a verbal consent was taken based on the participant's free will of participation before recruiting in the study.

Conflicts of interest

The authors have declared no conflict of interest.

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