



Is Brucellosis an emerging zoonotic infection in District Nowshera Pakistan? [A novel investigation involving descriptive analysis of laboratory reports (2019-2021)].

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ABSTRACT: A novel cross-sectional study was planned to determine the significant frequency, risk factors and clinical parameters of Brucellosis in people living in Nowshera, Pakistan from March 2019 to March 2021. A non-probability convenient sampling was performed targeting 617 individuals who were previously diagnosed with consistent fever and cough, abortion and infertility, musculoskeletal pain, headache and depression, and elevated serum Lactate dehydrogenase (LDH), elevated C-reactive Protein (CRP), and elevated Erythrocytes sedimentation rate (ESR). A closed-end questionnaire was filled out to gather information regarding different socio-demographics, assumed potential risk factors and clinical parameters of the participants. The serum samples of all participants were tested for IgG ELISA using (NovaLisa, GmbH, Germany). Descriptive statistics and statistical significance (null hypothesis) were analyzed via SPSS. 21. The entire frequency of human brucellosis found in our study was 14.1%. The highly significant sociodemographic factors were gender ($P=0.00001$), residency ($P=0.040$), and financial status ($P=0.007$), whereas the significant risk factors were animal exposure ($P=0.002$) and Brucella awareness ($P=0.003$). While the highly significant clinical complications were abortion and infertility ($P=0.00004$), elevated LDH ($P=0.0005$), elevated CRP ($P=0.00003$) and ESR ($P=0.0002$). The present seroprevalence of brucellosis in Nowshera is an alarming and serious public health concern. It is highly recommended to publicly provide a massive hygienic awareness and diagnostic services to explore the updates regarding the epidemiology of Brucellosis in the considered area for reasonable eradication.

Keyword: Brucellosis, zoonotic infection, Risk factors, Clinical Parameters, Nowshera, Pakistan.

INTRODUCTION

Brucellosis is one of the emerging zoonotic infections which is found across the globe. It is chiefly caused by a few members of the *Brucella* genus which infect both humans and animals (Xavier et al., 2010). Only *B. abortus*, *B. canis*, *B. suis*, and *B. melitensis* (excluding biovar-2) are potentially known for causing human infection among some of the twelve *Brucella* species (Agasthya et al., 2007, 2012; Njeru et al., 2016). Brucellosis is one of the occupational diseases that primarily affect humans who encounter animals or animals-associated products, especially in farming areas where many of the local inhabitants work in livestock-husbandry or terrestrial agriculture. Mostly, human brucellosis includes non-specific clinical signs that might be misinterpreted for other infections that cause fever. Human brucellosis is principally associated with headache, depression, weight loss, weakness, undulant fever, joint pain, contagious abortion, severe back pain, loss of appetite, elevated LDH, ESR and CRP, and night sweating (Swai and Schoonman, 2009). Patients with brucellosis have been found to have fibromyalgia, bone and joint infections, cardiomyopathy, hepatomegaly, and

lymphadenopathy (Roth et al., 2003; Ulu-Kilic et al., 2013).

Brucellosis is mainly spread by infected vital fluids of humans or animals such as blood, placental fluids, cervical mucus, vaginal fluid, semen, and an aborted fetus via infected animals to humans (Ducrotoy et al., 2014). Whereas bacterial transfer from person to person is uncommon, *Brucellae* has been demonstrated also to be transmitted by sexual intercourse and breastfeeding (Agashtya et al., 2012). Direct inoculation through lesions and wounds, skin abrasions, conjunctival infections, and inhaling the infected aerosols are all communal routes of infection (Herrick et al., 2014; Memish and Balkhy, 2004). The prominent risk factors associated with human brucellosis mainly include occupational contact with livestock and the ingestion of unpasteurized milk or milk products such as yoghurt, kefir, and soft cheese (Rahman et al., 2012).

Brucellosis constitutes a considerable hazard to global health, particularly in developing Middle Eastern and Mediterranean nations (Musallam et al., 2016; Nikokar et al., 2011; Vrioni et al., 2009). Due to a lack of awareness, the incidence of brucellosis is on the rise in Pakistan

(Saeed et al., 2020; Arif et al., 2017). In the previous decade, just a few research works on human brucellosis have been conducted in countable areas of Pakistan (Mukhtar, 2010). Most of the rural residents of Pakistan are traditionally linked with animal husbandry, livestock farming, and animal slaughtering, and lack advanced occupational biosafety familiarity and disease surveillance system (Jamil et al., 2021).

Similarly, the lack of a reliable diagnostic facility and research studies have failed to report Brucellosis in Nowshera, Pakistan. Hence, suitable research regarding the bio-risk status of this neglected infection can be valuable to plan infection prevention and control strategies. So, the present research study was envisioned to evaluate the significant frequency, risk factors and clinical parameters in District Nowshera, Khyber Pakhtunkhwa, Pakistan.

MATERIALS AND METHOD

Study area and design

This novel cross-sectional research was assumed from March 2019 to March 2022 to determine the significant frequency, risk factors and clinical parameters of Brucellosis in District Nowshera which is 1748 Km² (34°0'55"N 71°58'29"E) and present in

Khyber Pakhtunkhwa province in Pakistan. Most of the developing residents of the targeted area are laborers and are regularly associated with agriculture and livestock. While in the same area, the diagnostics tests for brucellosis are mostly outsourced because of lacking appropriate standard diagnostic facilities.

Inclusion and exclusion criteria

The sampling procedure adopted for this study was a nonprobability convenience technique. Inclusively, [n=617 (male=261 and female=356)] blood serum samples were collected from patients who were previously diagnosed with consistent fever and cough, musculoskeletal pain, headache and depression, abortion and infertility, elevated Lactate Dehydrogenase (LDH), C- Reactive Protein (CRP), and Erythrocytes sedimentation (ESR). While those with no above-mentioned clinical evidence were correspondingly excluded. All participants were asked to contribute to the projected study voluntarily during their inclusion. The blood samples from all individuals were collected after getting their verbal consent. Each individual was involved after clarifying the purpose of the study. A closed-end questionnaire was filled out before sample collection to gather the information regarding particularly

different socio-demographics, assumed potential risk factors and clinical history (Fig. 1, Table 1, Table 2, and Table 3).

The subjects' names and medical record numbers were not obtained due to the individual's privacy.

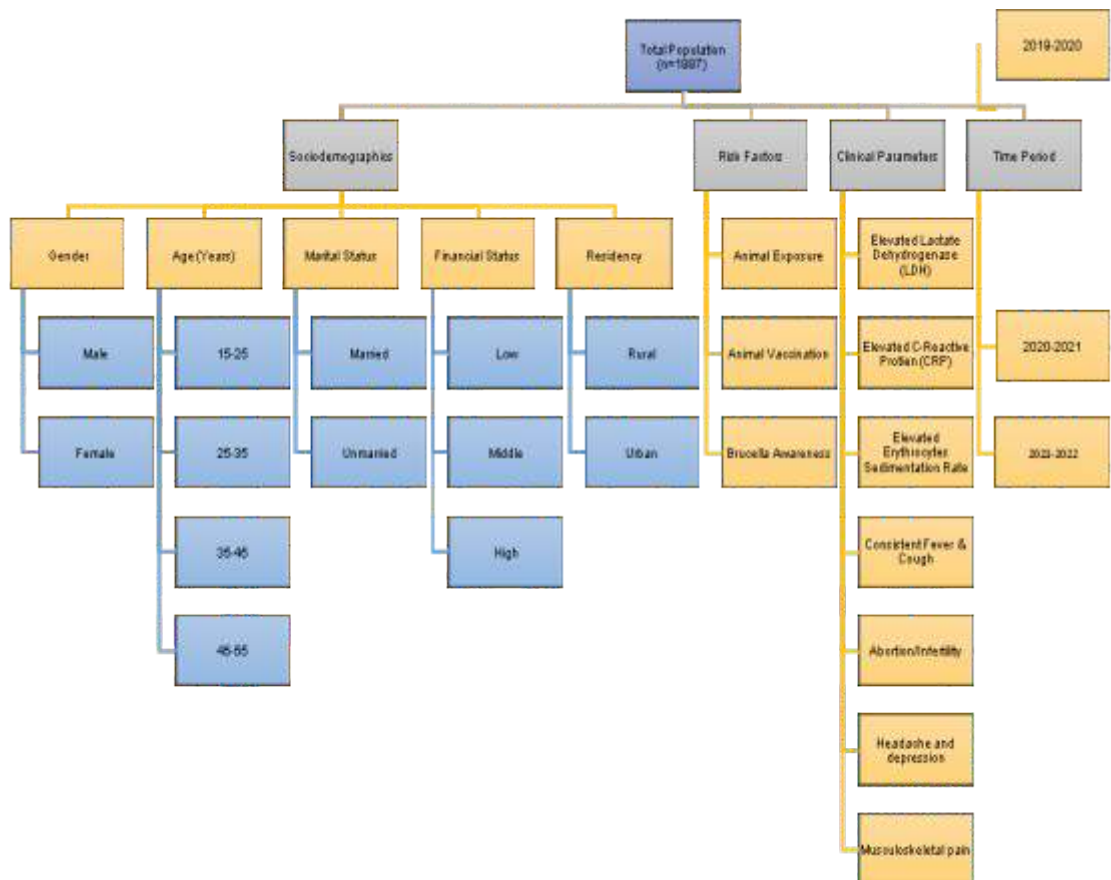


Fig. 1. The data flow diagram showed different sociodemographic, potential risk and clinical variables which were included in the study

Collection of samples

Aseptically, 5-millilitre blood samples were collected from each patient through venipuncture in properly sterile and disposable 10ml syringes with the help of trained staff. Then the samples were properly labelled with code, date, and place of collection. After collecting the samples, serum was

separated through centrifugation (3000 RPM) from all samples and the serum was stored in the freezer at 4°C till further process.

Confirmation by ELISA (Enzyme-Linked Immunosorbent Assay)

All of the serum samples were diagnosed for IgG ELISA using

(NovaLisa, GmbH, Germany *Brucella* IgG ELISA) kit as previously described. The ELISA procedures were followed according to the manufacturer's standardized instructions, with a cutoff value of 12u/ml for a positive antibody test. All serum samples were diluted at 1:10 for IgG detection, then 100ul of diluted serum was added to each well and the whole well-tray was incubated at 37°C for 1 hour. After washing, the conjugated enzyme reagent was added to each well and again incubated for 30 minutes. After the second washing round, the enzyme-substrate was added to each well for 20 minutes. The optical density (OD) for each well was evaluated at 450 nm after a stop buffer was added to each well. The OD values from the controls were utilized to create a standard curve. While the standard curve was used to calculate the value of the tested samples. Typically, the positive cases were considered for either one or both of *B. abortus*, and *B. melitensis* positive.

Statistical Analysis

The whole data of the research (statistical significance, and percentage frequencies) were investigated by SPSS version.21. All results were reflected as statistically significant at $P < .05$.

RESULTS

Frequency based on sociodemographic data

According to our study (Table 1), brucellosis is an exceedingly prevalent and hidden infection in Nowshera. Total (n=617) samples were processed, and it was experimentally observed that the entire percentage seroprevalence of human brucellosis in the study area was 14.10% (n=87/617). While the non-significant level of *Brucella* seroprevalence (16.2%, $P=0.051$) was seen during 2020 and 2021. Based on gender, the seroprevalence of human brucellosis was significantly highest in female individuals with 19.3% in contrast to males at 6.8% ($P=0.00001$) because the women of the developing communities of the same area are mostly exposed to non-vaccinated animals regularly. Among the different age groups studied, the highest level of *Brucella* prevalence was detected in the age group ranging 25-35 years with 16.4% followed by 15.6% in the 35-55 years age group, while the least prevalence of 13.5% was detected in 15-25 years age group ($P=0.36$). Besides, we discovered the significantly highest *Brucella* prevalence of 16.4% in rural residents of Nowshera, which is followed by 10.5% in urban residents

(with $P=0.040$). Whereas a non-significant highest seroprevalence of 14.5% was observed in a married group of participants followed by 11.5% prevalence in unmarried individuals

($P=0.442$). Further, based on financial status, we noted a significant prevalence of 18.1% in people of low income followed by 9.7% in people having middle-income status ($P=0.006$).

Table 1: Frequency of Brucellosis based on different sociodemographic data from 2019-2021

Demographics	Total tested (n)	Seronegative (n)	Seropositive (n)	Percentage Frequency (%)	Chi-square value	Significance (P value)
Overall	617	530	87	14.1	---	---
Gender					19.38	0.00001
Male	261	243	18	6.8		
Female	356	287	69	19.3		
Age group (years)					3.20	0.36
15-25	81	70	11	13.5		
25-35	285	238	47	16.4		
35-45	219	195	24	10.9		
45-55	32	27	05	15.6		
Residence					4.21	0.040
Urban	246	220	26	10.5		
Rural	371	310	61	16.4		
Marital status					0.58	0.442
Married	522	446	76	14.5		
Unmarried	95	84	11	11.5		
Financial status					9.92	0.007
Low	337	276	61	18.1		
Middle	195	176	19	9.7		
High	85	78	07	8.2		
Time (Years)					5.94	0.051
2019-2020	276	233	43	15.5		
2020-2021	209	175	34	16.2		
2021-2022	132	122	10	7.5		
The result is statistically significant at $p < .05$.						

Frequency based on assumed potential risk factors

Out of the 617 participants, a highly significant prevalence of 16.3% (n=79/404) was found in those who were consistently exposed to animals (including buffaloes, cows, dogs, goats, and sheep) followed by 5.9% in those who were not exposed to animals (**P=0.002**). The chief purpose of their animal’s exposure was milk and dairy products consumption and business, meat source, and agriculture. The reason behind this was found that the rural

inhabitants of Nowshera sell meat from slaughtering activities in public to provide a protein source to the residents. Based on animal vaccination, 16.3% (n=81/495) of individuals were a victim of Brucellosis who did not vaccinate their animals against any zoonotic disease (including brucellosis) which was statistically non-significant (**P=0.9**). Whereas 17.8% (n=85/477) of individuals were prone to Brucellosis who had no awareness of Brucellosis which was also statistically significant (**P=0.03**) (Table 2).

Table 2: Frequency of Brucellosis based on different assumed potential risks

Potential Risk	Total tested (n)	Seronegative (n)	Seropositive (n)	Percentage Frequency (%)	Chi- square Value	Significance (P value)
Animal exposure					9.34	0.002
No	134	126	08	5.9		
Yes	483	404	79	16.3		
Vaccinated animals					0.01	0.9
No	576	495	81	16.3		
Yes	41	35	06	14.6		
Brucella Awareness					4.55	0.03
No	562	477	85	17.8		
Yes	55	53	02	3.77		

The result is statistically significant at $p < .05$.

Frequency based on different clinical parameters

It was also discovered that the infected individuals were significantly associated with several abnormal

clinical conditions. Among all the observed clinical parameters, abortion and infertility (26.7%, **P=0.00004**), elevated LDH (23.3%, **P=0.00005**), elevated CRP (53.8%, **P=0.00003**), and

elevated ESR (17.9%, **P=0.0002**) were significantly associated with the *Brucella* seropositive patients (Table 3). Whereas participants with consistent fever and cough (P=0.115), headache

and depression (P=0.12), and musculoskeletal pain (P=0.91) were non significantly correlated to brucellosis (Table 3).

Table 3: Frequency of Brucellosis based on different clinical parameters

Clinical Parameters	Total tested (n)	Seronegative (n)	Seropositive (n)	Percentage Frequency (%)	Chi-square value	Significance (P value)
Consistent Fever and Cough					2.47	0.115
Yes	603	520	83	13.7		
No	14	10	04	28.5		
Headache and depression					2.41	0.12
Yes	19	14	05	26.3		
No	598	516	82	13.7		
Musculoskeletal pain					0.01	0.91
Yes	132	113	19	14.3		
No	485	417	68	14.0		
Abortion and infertility					16.9	0.00004
Yes	105	77	28	26.7		
No	512	453	59	11.5		
Elevated LDH (Lactate Dehydrogenase)					11.8	0.0005
Yes	133	102	31	23.3		
No	484	428	56	11.6		
Elevated CRP (C-Reactive Protein)					17.3	0.00003
Yes	13	06	07	53.8		
No	604	524	80	13.2		
Elevated ESR (Erythrocytes sedimentation)					13.1	0.0002
Yes	389	319	70	17.9		
No	228	211	17	7.4		
The result is statistically significant at $p < .05$.						

DISCUSSION

In general, few studies have been published in Pakistan focused on

brucellosis in humans (Arif et al., 2017). Furthermore, studies featured women who had abortions in the past, and clinical indicators are rare in Pakistan (Yousaf et al., 2021). Thus, researchers and policymakers will gain fresh data and information concerning human brucellosis in Pakistan as a result of this study.

However, human brucellosis (commonly known as contagious abortion or undulant fever) has been broadly considered in domesticated or pet animals in Punjab province (Hussain et al., 2020; Jamil et al., 2020), and less is documented about the infections in humans. Serology and PCR were used in a few studies, whereas culture and subsequent identification were used in almost none. ELISA detected 21.7% of 360 venipuncture blood samples obtained from employees at four slaughtering cells in the Lahore district of Punjab province (Mukhtar, 2010). Using ELISA, a percentage seroprevalence of 27% was discovered in 304 lady patients from a high-risk cohort in Malakand, Khyber Pakhtunkhwa province (Niaz et al., 2021). Females aged 21–30 years old had a higher prevalence (32.25%) than males aged 21–30 years old. In Peshawar (the capital of Khyber Pakhtunkhwa province), seroprevalence

was 32.90% across the hospitalized patients in the same district (Shahid et al., 2014). Also, 5.8% of the 429 blood samples obtained from pregnant women at Benazir Bhutto Hospital Rawalpindi were serologically positive (Ali et al., 2016).

In the research study group of seventy patients at Abbottabad's Teaching Hospital, 49 were seropositive (Malik et al., 2018). The bulk of the patients (n=35) were between the ages of 21 and 40. RBPT and *B. abortus* PCR tests were positive in 10.1% and 5.8% of acute febrile cases, respectively, in Islamabad and Rawalpindi clinics (Saddique et al., 2019). In the Potohar region in northeastern Pakistan, 6.9% of the total 262 blood serum samples obtained from various occupational employees were found to be potentially infectious. RT-PCR revealed that all patients were infected with *B. abortus* (Ali et al., 2013).

People who ate raw meat and dairy had a greater rate of infection. The lower occurrence of 6.9% in the Potohar region in northeast Pakistan (Mukhtar, 2010) and 9.33% in Azad Jammu & Kashmir (Arif et al., 2019) could be enlightened by less animal contact and less unpasteurized milk consumption in these locations. Because of variances in each test's specificity and sensitivity, the

diverse serological tools utilized could ultimately be a cause for differing prevalence and severity.

Brucellosis has been diagnosed using a variety of serological and molecular approaches (Di Bonaventura et al., 2021; Poester et al., 2010). When a positive result is obtained, however, quantitative ELISA is a highly accurate and precise approach for promptly diagnosing and classifying *Brucella* species in the targeted samples. In hospital admitted patients with medical indications but negative RBPT diagnostic results, the IgG and IgM ELISA test results are substantial, enabling for initial and quick serological confirmation of contagious abortion in humans by quantification of immunological load. When compared to females, males had the lowest seropositivity (clinical cases 2.43%) in our study. In previous research, women (37%) had greater prevalence rate rates than diagnosed men (24.2%) in hospital-admitted patients in Peshawar (Shahid et al., 2014). Because of their everyday tasks, female and housewife employees in less populated regions have greater direct contact with their livestock, resulting in higher seroprevalence rates in females (Yousaf et al., 2021). They sometimes assist animals at delivery and eradicate aborted fetuses overlooking

any precautions, putting them at risk from extended contact with infected animals. As a result, activities in places where animal-born brucellosis is uncontrolled raise the chance of contracting the disease. Because human *Brucella* infection is an occupational illness, people of age 50 years are more likely to contract it.

In Punjab, a survey of brucellosis knowledge among small dairy farm employees was undertaken in 2015. According to the survey, 97% of farmers are unaware of brucellosis as well as its transmission routes (Arif et al., 2017). In Pakistan, cattle and buffaloes are the most common livestock and also the most common source of milk. *B. abortus* is the most frequent source of bovine brucellosis (Soomro et al., 2014). This helps to explain why *B. abortus* was found in all the human instances in this investigation. In 420 herds across seven areas in Pakistan, bovine brucellosis was researched, and the incidence rate in herds was 16.2% (Hussain et al., 2020). At institutional-owned animal farms in Punjab (a highly populated Pakistani province), the percentage seroprevalence of 3.9% and 3.3% were documented by using RBPT and ELISA, respectively (Jamil et al., 2020).

CONCLUSION

Unseen life threats of human brucellosis are predominantly found in Nowshera, Khyber Pakhtunkhwa, Pakistan. It was found that the people who were engaged with livestock were significantly more vulnerable to Brucellosis. It is keenly advised to publicly provide a massive hygienic awareness and diagnostic services to explore the epidemiology of *Brucella* in the considered area as well as in the neighboring territories for rational infection eradication.

Conflicts of interest

The authors declare no conflict of interest. All authors have participated equally.

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