



Epidemiological Assessment of Human Brucellosis among Females in Malakand District, Pakistan Using Rapid Diagnostic Testing

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ABSTRACT

Brucellosis is a significant zoonotic disease caused by bacteria of the genus *Brucella*, affecting both animals and humans. The disease remains endemic in many developing countries where livestock farming and close human-animal interaction are common. Limited diagnostic facilities and lack of awareness often lead to underreporting of cases, particularly in rural communities. The present study aimed to investigate the distribution of *Brucella* infection among female participants in Malakand district using rapid diagnostic testing. A cross-sectional study was conducted involving 160 female participants from different areas of Malakand district, Khyber Pakhtunkhwa, Pakistan. Blood samples were collected under aseptic conditions and tested using a Rapid Diagnostic Test (RDT) for the detection of antibodies against *Brucella abortus* and *Brucella melitensis*. Demographic information including age, residential area and test date was recorded. Data were organized in Microsoft Excel and graphical analysis was performed to evaluate age-wise, area-wise and temporal distribution of cases. Age distribution showed the highest number of cases in the 21-30-year group (93 cases), followed by 11-20 years (40 cases) and 31-40 years (27 cases). Spatial analysis indicated that Batkhela recorded the highest number of cases (35), followed by Chakdara (21) and Thana (20). Monthly analysis revealed a peak in August with 26 cases, indicating seasonal variation in infection occurrence. The study demonstrates that brucellosis remains a notable public health concern in rural areas with strong livestock interaction. Strengthening surveillance, promoting safe animal handling practices and improving public awareness may help reduce disease transmission.

1. INTRODUCTION

Zoonotic diseases remain a significant global public health concern because they affect both human and animal populations and can spread rapidly across communities (Esposito et al., 2023). Among these diseases, brucellosis is one of the most widespread bacterial zoonoses, particularly in regions where livestock farming forms a major part of the economy. The disease is caused by bacteria of the genus *Brucella* and is primarily transmitted to humans through direct contact with infected animals, consumption of unpasteurized dairy products, or exposure to contaminated animal products (Islam et al., 2023). Brucellosis affects millions of people worldwide and continues to be underdiagnosed in many developing

countries due to limited diagnostic facilities and lack of awareness (Samadi et al., 2024). Its symptoms are often nonspecific, including fever, fatigue and joint pain, which makes early detection difficult and allows the disease to persist as a chronic public health issue (Ulu Kilic et al., 2013).

Brucella species are small, Gram-negative, facultative intracellular bacteria that primarily infect domestic animals such as cattle, goats and sheep. Among the various species, *Brucella abortus* and *Brucella melitensis* are considered the most important pathogens responsible for human brucellosis (Adem & Duguma, 2020). These organisms have the ability to survive and replicate within host cells, allowing them to evade immune responses and

establish persistent infections (Thakur et al., 2019). Infection in animals often leads to reproductive problems, including abortions, infertility and reduced milk production, causing substantial economic losses for livestock farmers (Deka et al., 2021). Humans become accidental hosts when they are exposed to infected animals or animal products during farming, veterinary practices, or food consumption. Because of its occupational risk, brucellosis is commonly observed among farmers, butchers, veterinarians and laboratory workers who frequently interact with potentially infected livestock (Nicoletti, 2020).

Brucellosis remains highly prevalent in many developing countries, including those in South Asia, the Middle East and parts of Africa (Bagheri Nejad et al., 2020). In these regions, traditional livestock farming practices, inadequate veterinary surveillance and limited access to diagnostic facilities contribute to the persistence of the disease (Lane et al., 2025). Pakistan is considered an endemic country for brucellosis, particularly in rural areas where animal husbandry is a primary source of livelihood (Awais et al., 2024). The close interaction between humans and livestock increases the risk of transmission, especially where pasteurization of milk and dairy products is not consistently practiced (Holzhauer & Wennink, 2023). Despite its significance, brucellosis is often underreported due to insufficient screening and diagnostic limitations. Consequently, many infected individuals remain undiagnosed or misdiagnosed, which further complicates disease control and prevention strategies in affected communities (Pellejero-Sagastizábal et al., 2025).

Accurate and timely diagnosis plays a critical role in controlling brucellosis and preventing its spread among both humans and animals (Qureshi et al., 2023). Several laboratory techniques are used for the detection of *Brucella* infection, including culture methods, polymerase chain reaction assays and serological tests (Di Bonaventura et al., 2021). However, many of these techniques require advanced laboratory infrastructure, trained personnel and considerable time to produce results (Salman et al., 2019). Rapid diagnostic tests have therefore emerged as a practical alternative, particularly in resource-limited settings (Heidt et al., 2020). Rapid diagnostic tests provide quick results, are relatively simple to perform and can be used in field conditions or small laboratories (Wilson, 2013). These advantages make them valuable tools for early screening and surveillance of brucellosis in endemic regions where conventional diagnostic facilities may not always be readily available.

Given the public health importance of brucellosis and the need for efficient diagnostic approaches, epidemiological investigations are essential for understanding the distribution of the disease in specific populations. The present study focuses on the detection of *Brucella* species using rapid diagnostic testing among female participants from the Malakand district. By examining demographic factors such as age and residential area, this research aims to provide insight into the occurrence of *Brucella* infection within the study population. Identifying the presence of *Brucella abortus*

and *Brucella melitensis* through rapid diagnostic methods can contribute to improved disease surveillance and management strategies. The findings of this study may also help highlight the importance of routine screening and early detection to reduce the burden of brucellosis in livestock-dependent communities.

2. MATERIAL AND METHODS

1. Study Area and Site Selection

The present study was conducted in the Malakand district of Khyber Pakhtunkhwa, Pakistan, an area where livestock farming is a common livelihood activity. The district was selected due to its extensive interaction between humans and domestic animals such as cattle, goats and sheep, which increases the risk of zoonotic infections including brucellosis. Rural and peri-urban areas within the district were included in the study to capture potential exposure patterns associated with livestock handling and dairy consumption. Sampling locations were chosen based on accessibility, willingness of participants to provide samples and the presence of livestock rearing practices in the community. This site selection was intended to represent populations with a higher probability of exposure to *Brucella* species.

2. Study Design and Population

A cross-sectional study design was used to investigate the occurrence of *Brucella* infection among female participants in the selected study area. The study population consisted of females residing in Malakand district who voluntarily participated during the sampling period. Demographic information including age, area of residence and test date was recorded for each participant. The age of participants was documented in years to assess the distribution of infection across different age groups. A total of 160 samples were included in the study dataset. The study focused on females due to their significant involvement in livestock management and household dairy handling activities in rural communities.

3. Sample Collection

Blood samples were collected from participants following standard clinical procedures. Sterile disposable syringes were used to draw venous blood under aseptic conditions. After collection, samples were carefully transferred into labeled tubes containing appropriate anticoagulant or serum separation materials. Each sample was assigned a unique identification number to ensure traceability and proper data management. Samples were then transported to the laboratory under controlled conditions to maintain sample integrity. Basic demographic information including participant age, gender, district and residential area was recorded alongside each sample to facilitate epidemiological analysis and interpretation of results.

4. Detection of *Brucella* Species Using Rapid Diagnostic Test (RDT)

Detection of *Brucella* infection was carried out using a Rapid Diagnostic Test (RDT), which is a serological method designed to identify antibodies against *Brucella* species in human serum. The test procedure was performed according to the manufacturer's instructions. A

small volume of serum was applied to the test device followed by the addition of a buffer solution. The test results were interpreted based on the appearance of colored lines within the specified reaction time. The RDT allowed the detection of antibodies associated with *Brucella abortus* and *Brucella melitensis*, which are the most common species responsible for human brucellosis. Positive and negative results were recorded for each sample.

5. Data Recording and Management

All collected information and laboratory results were systematically recorded in a structured data sheet. The dataset included variables such as sample number, gender, age in years, district, residential area, date of testing, detected bacterial species and RDT test results. Data were carefully reviewed to ensure accuracy and completeness before analysis. Each entry was checked to avoid duplication or missing information. Proper labeling and coding of variables were maintained to facilitate statistical processing. The organized dataset allowed efficient evaluation of demographic characteristics and infection status among the study participants.

6. Statistical Analysis

The collected data were organized and analyzed using Microsoft Excel. Descriptive analysis was performed to summarize demographic variables including age, district and residential area of the participants. The results of the Rapid Diagnostic Test (RDT) were categorized as positive or negative and recorded for each sample. Graphical representations were generated to illustrate the distribution of *Brucella* infection among the study population. Different types of graphs were created to visualize patterns related to age distribution, bacterial species detected and area-wise occurrence of infection. These graphs were used to present the results in a clear and understandable manner, allowing easier interpretation of trends and variations within the dataset. The graphical analysis helped in highlighting the overall occurrence of *Brucella* infection among the sampled participants.

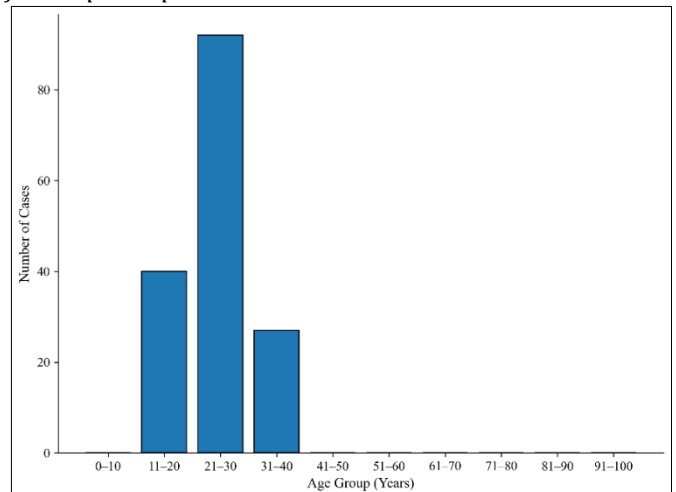
3. RESULTS

a. Age Group Distribution

Age-wise distribution of confirmed brucellosis cases revealed a clear concentration in young adults within the studied population of Malakand district (Fig. 1). The highest number of cases was recorded in the 21-30 years age group with 93 cases, representing the dominant proportion of the dataset. The second most affected group was 11-20 years with 40 recorded cases. Individuals aged 31-40 years accounted for 27 cases, indicating comparatively lower occurrence than younger adults. No positive cases were detected in the remaining age groups, including 0-10, 41-50, 51-60, 61-70, 71-80, 81-90 and 91-100 years. This distribution suggests that exposure risk is concentrated primarily among active young females involved in livestock handling and dairy related household activities within rural communities of the study area overall.

Figure 1

Age-wise distribution of confirmed brucellosis cases among female participants in Malakand district.

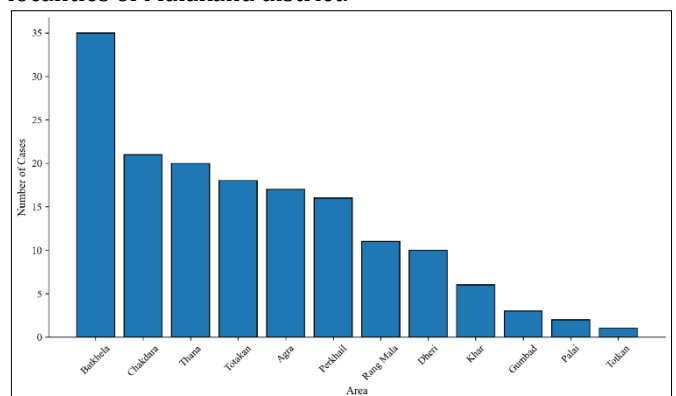


b. Area-wise Distribution

Area-wise distribution of detected brucellosis cases demonstrated clear spatial variation across different localities of Malakand district (Fig. 2). Batkhela showed the highest number of cases with 35 confirmed infections. Chakdara recorded 21 cases, followed by Thana with 20 cases. Totakan contributed 18 cases, while Agra reported 17 cases. Perkhail accounted for 16 cases, indicating moderate occurrence in this locality. Rang Mala showed 11 cases, whereas Dheri documented 10 cases. Lower frequencies were observed in Khar with 6 cases, Gumbad with 3 cases, Palai with 2 cases and Totkan with only 1 recorded case. This geographic pattern indicates heterogeneous distribution of infection possibly linked to livestock density, animal husbandry practices, human-animal contact intensity and dairy consumption habits within different rural settlements of Malakand.

Figure 2

Area-wise distribution of brucellosis cases across different localities of Malakand district.



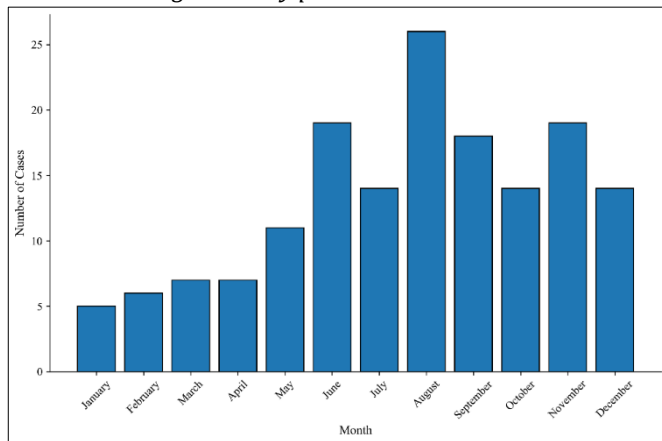
c. Monthly Distribution

Monthly distribution of confirmed brucellosis cases revealed noticeable temporal variation throughout the study period (Fig.). January recorded 5 cases, while February showed 6 cases. March documented 7 cases and April also reported 7 cases. A marked increase was observed in May with 11 cases. The number further rose

in June to 19 cases, followed by 14 cases in July. The highest monthly occurrence was recorded in August with 26 cases. September documented 18 cases, whereas October showed 14 cases. November again increased to 19 cases and December reported 14 cases. This seasonal pattern suggests higher transmission during late summer months possibly associated with intensified livestock breeding, milk handling, animal parturition events and increased human exposure to infected animal products within rural communities.

Figure 3

Monthly distribution of brucellosis cases showing seasonal variation during the study period.

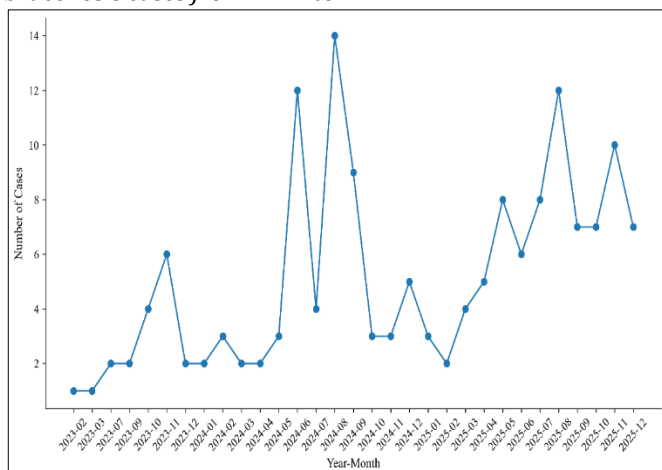


d. Year-Month Trend (120 words)

Year-month trend analysis demonstrated gradual fluctuations in the occurrence of brucellosis cases across the surveillance period (Fig. 4). In 2023, February and March each recorded 1 case, while July and September reported 2 cases each. October showed 4 cases and November increased to 6 cases, followed by 2 cases in December. During 2024, January recorded 2 cases, February 3, March 2, April 2 and May 3 cases. A substantial rise occurred in June with 12 cases, July 4, August 14, September 9, October 3, November 3 and December 5 cases. In 2025, January recorded 3 cases, February 2, March 4, April 5, May 8, June 6, July 8, August 12, September 7, October 7, November 10 and December 7 cases respectively overall.

Figure 4

Year-month timeline illustrating temporal trends of brucellosis cases from 2023 to 2025.



4. DISCUSSION

Brucellosis is a globally important zoonotic disease that affects both humans and animals, particularly in regions where livestock farming plays a central socioeconomic role. The disease remains endemic in many developing countries, including Pakistan, where close human-animal interaction and consumption of unpasteurized dairy products facilitate transmission (Aslam et al., 2023). Epidemiological investigations have shown that brucellosis is frequently underreported because its clinical symptoms resemble other febrile illnesses and diagnostic facilities are limited in many rural areas (Qureshi et al., 2023). Pakistan possesses a large livestock population and animal husbandry contributes significantly to rural livelihoods, which increases the potential for zoonotic transmission (Raza et al., 2023). Consequently, continuous surveillance and epidemiological studies are necessary to understand disease patterns and risk factors. Such investigations help identify vulnerable population groups and guide the development of targeted prevention and control strategies in endemic regions.

The results of the present study demonstrated a clear demographic pattern in the distribution of brucellosis cases among the studied female population of Malakand district. Age-wise analysis showed that the highest number of cases occurred in the 21-30-year age group with 93 cases, followed by 40 cases in the 11-20-year group and 27 cases in the 31-40-year group, while no cases were recorded in other age categories. Area-wise distribution indicated that Battkhelda reported the highest number of cases (35), followed by Chakdara (21) and Thana (20), whereas minimal frequencies were observed in Palai (2) and Totakan (1). Temporal analysis also revealed seasonal variation, with the highest number of cases in August (26) and elevated numbers in June and November (19 cases each), suggesting increased transmission during warmer months.

These findings are consistent with several previous epidemiological studies conducted in endemic regions. Earlier investigation by Pereira et al., (2020) have reported that brucellosis is more common among individuals in the active adult age groups, particularly between 20 and 50 years, largely due to occupational exposure to livestock and animal products (Pereira et al., 2020). Similarly, Niaz et al., 2021 reported in Pakistan and other rural settings have demonstrated higher disease occurrence among populations that maintain close contact with animals or handle dairy products during routine household activities (Niaz et al., 2021). The age-group pattern observed in the present study, with the highest number of cases in the 21-30-year category, therefore aligns with the epidemiological trends reported in previous research, suggesting that young adults represent an important risk group for brucellosis transmission (Assafi et al., 2019).

Seasonal and spatial patterns observed in this study also correspond with previously reported epidemiological trends of brucellosis. Bülbül & Ataman (2009) have documented that the disease demonstrates clear seasonal variation, with increased incidence during spring and summer months, which are associated with livestock breeding, calving and increased handling of animal

products (Bülbül & Ataman, 2009). The peak observed in August in the present study may therefore reflect intensified animal-human contact during these periods. Additionally, higher case frequencies in specific localities such as Batkhela may be related to differences in livestock density, animal husbandry practices and public awareness regarding zoonotic diseases (Khan et al., 2025). These findings highlight the importance of strengthening surveillance programs, promoting safe dairy consumption practices and improving community awareness to reduce the burden of brucellosis in rural populations (Dadar et al., 2021).

5. CONCLUSION

The present study highlights the occurrence and

distribution of brucellosis among female participants in Malakand district using rapid diagnostic testing. Age-based analysis indicated that young adults, particularly the 21-30-year age group, were the most affected population. Spatial distribution showed higher case frequencies in areas such as Batkhela, Chakdara and Thana, suggesting localized variations possibly associated with livestock exposure and husbandry practices. Temporal analysis further demonstrated seasonal variation, with the highest number of cases recorded during late summer months. These findings emphasize the importance of improved surveillance, early diagnostic screening and community awareness programs to reduce transmission risks and support effective management of brucellosis in livestock-dependent rural communities.

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