Volume 3, Number 3, 2026
Pages 1699-1709
Bioscience Insights
ISSN: 3080-3489 (Print)
ISSN: 3080-3497 (Online)
BiosciInsights.com

ISOLATION, IDENTIFICATION, AND ANTIBIOGRAM PATTERN OF BRUCELLA ABORTUS BACTERIA FROM RAW MILK AND ASSESSMENT OF POTENTIAL HUMAN RISK FACTORS IN TEHSIL PAHARPUR, DISTRICT DERA ISMAIL KHAN

Muhammad Umar[1], Khalid Muhammad[2], Muhammad Tariq[3], Hijab Iqra Khan[4], Saqib Ali Rustam[5], Muhammad Noman Hassan Khan[6], Tayyab Khan[7], Syed Mubashir Hassan[8], Noor Ul Ain[9], Shahzada Khurram Adrian Shah[10], Atta Ur Rehman[*11]

*[1,2,4,6,*7,11]Institute of Microbiology, Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan*
[3,5,8]Faculty of Veterinary and Animal Sciences, Gomal University, Dera Ismail Khan
[9]Institute of food Science and Nutrition, Gomal University, Dera Ismail Khan
[10]Department of Clinical Studies Faculty of Veterinary and Animal Sciences, UVAS SWAT

https://doi.org/10.5281/zenodo.24156491

Article Information

Article History

Received:	June 19, 2026
Revised:	August 19, 2026
Accepted:	September 07, 2026
Available Online:	October 31, 2026

Keywords:
Brucella abortus; *raw milk; antimicrobial resistance; zoonotic disease; risk factors; Paharpur*

Copyright @Author
Corresponding Author: *
Atta Ur Rehman

Abstract

Brucella abortus is an important zoonotic, Gram-negative bacterium responsible for brucellosis, a disease that can be transmitted from infected animals to humans through direct contact with infected animals or consumption of contaminated, unpasteurized milk and dairy products. The present study was conducted in Tehsil Paharpur, District Dera Ismail Khan to investigate the occurrence of Brucella *in raw milk samples, assess the antimicrobial resistance profile of the recovered isolates, and identify potential risk factors associated with human exposure. A total of 80 raw milk samples were collected from different areas of Tehsil Paharpur and subjected to bacteriological examination. Initial examination identified 57 samples with suspected bacterial growth, of which 48 were subsequently reported as positive following further identification procedures, representing an overall positivity of 60%. The antimicrobial susceptibility profile of the recovered isolates was evaluated against eight antibiotics. The highest reported resistance was observed against ampicillin (66.66%), whereas the lowest resistance was recorded against tetracycline (30%). Potential risk factors for human exposure included inadequate vaccination practices, lack of personal protective equipment during animal handling and laboratory-related activities, direct contact with potentially infected animals, and improper disposal of animal waste and reproductive materials. The findings highlight the potential public health importance of* Brucella

contamination in raw milk and the need for improved livestock health management, milk hygiene, occupational safety, and public awareness. Further molecular confirmation and representative epidemiological investigations are recommended to establish the identity of the isolates and determine the wider distribution of brucellosis in the study area.

INTRODUCTION

Brucellosis is one of the most important neglected zoonotic diseases worldwide, affecting both human and animal populations and causing substantial public health and economic consequences. It is caused by bacteria belonging to the genus *Brucella*, which are Gram-negative, non-motile, non-spore-forming, facultative intracellular organisms. Among the recognized species, *Brucella abortus, Brucella melitensis*, and *Brucella suis* are the major causes of human brucellosis, with *B. abortus* being primarily associated with cattle and buffaloes. The disease is transmitted from infected animals to humans through direct contact with contaminated animal tissues, blood, placental materials, uterine secretions, and consumption of contaminated animal products, particularly raw milk and dairy products. Brucellosis remains endemic in many developing countries, particularly in regions where livestock farming is an important component of the agricultural economy and preventive veterinary services are inadequate (Jamil et al., 2021; Qureshi et al., 2023).

Brucellosis has been recognized as a zoonotic disease for more than a century. Historically, the organism responsible for Malta fever was first isolated in 1887 by David Bruce from the spleen of an infected soldier in Malta and was initially named Micrococcus melitensis. Subsequently, Bernhard Bang identified the organism responsible for bovine abortion, which was later designated *Brucella* abortus. Further investigations established the relationship between different *Brucella* species and their respective animal hosts, providing the foundation for understanding the epidemiology and transmission of the disease. Although substantial progress has been made in the diagnosis, prevention, and control of brucellosis, it continues to pose a major challenge in livestock-producing countries because of persistent animal reservoirs, inadequate surveillance, and continuous exposure of humans to infected animals and contaminated animal products (Qureshi et al., 2023).

In animals, brucellosis primarily affects the reproductive system and is commonly associated with abortion, retained placenta, infertility, orchitis, reduced milk production, and the birth of weak offspring. Infected animals may shed large numbers of bacteria through reproductive discharges, particularly during abortion and parturition, as well as through milk. Consequently, contaminated raw milk represents an important potential source of infection for consumers. In humans, the disease may cause undulant fever, excessive sweating, fatigue, headache, muscle pain, joint pain, and general weakness. In some cases, infection may become chronic and result in serious complications, including osteoarticular involvement, endocarditis, neurological disorders, and reproductive complications. The nonspecific clinical manifestations of human brucellosis frequently create diagnostic difficulties, particularly in endemic regions where the disease may be confused with other febrile illnesses (World Health Organization [WHO], 2020; Qureshi et al., 2023).

The consumption of unpasteurized milk and dairy products is considered one of the major routes of human brucellosis transmission. In developing countries, traditional milk production, informal marketing systems, inadequate refrigeration, and insufficient implementation of milk pasteurization practices increase the possibility of exposure to foodborne pathogens. Pakistan is an important livestock-producing country where milk and dairy products contribute significantly to household nutrition and the national economy. However, a considerable proportion of milk is marketed through informal supply chains, particularly in rural areas, where appropriate hygienic handling and heat-treatment practices may not always be followed. Previous research has identified brucellosis as an important public health and veterinary concern in Pakistan, with evidence of infection in cattle, buffaloes, sheep, goats, and humans. The close interaction between livestock and rural communities, together with the consumption of raw

milk and inadequate occupational protective measures, increases the potential for disease transmission (Jamil et al., 2021).

Human brucellosis is particularly associated with occupational and behavioural risk factors. Farmers, livestock handlers, veterinarians, dairy workers, butchers, and abattoir workers are among the populations at increased risk because of their frequent exposure to infected animals and their biological materials. In Pakistan, a cross-sectional study conducted in Punjab reported a human brucellosis seroprevalence of 13.13% and identified several associated risk factors, including farming as an occupation, keeping animals at home, slaughtering animals, and consuming raw milk. Lack of education and inadequate awareness regarding disease transmission and preventive practices may further increase the likelihood of infection. These findings emphasize the importance of investigating local epidemiological factors and strengthening public awareness, food safety, occupational hygiene, and veterinary surveillance through an integrated One Health approach (Nawaz et al., 2021).

Accurate diagnosis and identification of *Brucella* species are essential for understanding disease distribution, identifying potential sources of infection, and developing effective control strategies. Conventional serological methods, including the Rose Bengal plate test and enzyme-linked immunosorbent assay, are widely used for screening and epidemiological investigations. Molecular techniques, particularly polymerase chain reaction (PCR), provide additional opportunities for rapid and specific detection of *Brucella* DNA. Bacterial isolation and identification can provide definitive evidence of viable organisms; however, culture-based investigations involving pathogenic *Brucella* require specialized containment facilities and stringent biosafety procedures because of the organism's high infectivity and potential for aerosol transmission. Therefore, suspected *Brucella* specimens and cultures must be managed by appropriately trained personnel in suitably equipped reference laboratories (Di Bonaventura et al., 2021).

Antimicrobial treatment is an important component of human brucellosis management; however, successful treatment generally requires prolonged combination therapy because of the intracellular nature of the organism and the possibility of relapse. The selection of appropriate antimicrobial agents is therefore important for effective clinical management. Nevertheless, antimicrobial susceptibility testing of *Brucella* isolates is not routinely recommended in ordinary diagnostic laboratories because of significant occupational hazards, limited standardization of testing procedures, and the recognized susceptibility of most isolates to recommended combination therapies. When susceptibility investigations are scientifically justified, they should be conducted using validated methods in appropriately equipped reference laboratories rather than relying on unvalidated disc-diffusion results (Dadar et al., 2024).

Despite previous investigations into animal and human brucellosis in Pakistan, important geographical and epidemiological gaps remain, particularly concerning localized disease occurrence, contaminated dairy products, and exposure-related risk factors in rural communities. Tehsil Paharpur, District Dera Ismail Khan, is an important area for livestock farming and milk consumption, where close human–animal interaction may create opportunities for zoonotic disease transmission. However, detailed information concerning the occurrence of *Brucella* in raw milk, species-specific identification, and associated human exposure factors in this particular study area remains limited. The absence of sufficient localized information may restrict the development of effective disease surveillance, preventive interventions, and public health awareness programmes.

Therefore, the present study is designed to investigate the occurrence of *Brucella* abortus associated with raw milk in Tehsil Paharpur, District Dera Ismail Khan, and to assess the potential public health implications of milk-borne transmission. The study will focus on laboratory-based detection and identification of the target pathogen, evaluation of antimicrobial susceptibility where scientifically and biosafely justified, and assessment of potential risk factors associated with human exposure. The findings are expected to contribute to the existing epidemiological knowledge of brucellosis in the study area and provide evidence to support improved milk hygiene, appropriate pasteurization practices, occupational protection, livestock disease surveillance, and community awareness.

Materials and methods

Study Area and Study Design

A cross-sectional study was conducted in Tehsil Paharpur, District Dera Ismail Khan, Khyber Pakhtunkhwa to investigate the occurrence of *Brucella abortus* in raw milk samples collected from livestock and assess the associated risk factors for human exposure. Tehsil Paharpur was selected because of its predominantly rural and agricultural economy, where livestock farming represents an important source of livelihood. The area is characterized by extensive livestock production and close interactions between humans and domestic animals. Cattle and buffaloes are the predominant dairy animals, while sheep and goats are also commonly reared by local communities. These livestock production practices, together with the consumption of raw milk and traditional dairy products, make the area relevant for investigating the potential transmission of zoonotic pathogens.

Sample Size and Sampling Strategy

A total of 80 raw milk samples were included in the study. Samples were collected from four livestock species, including cattle (n = 25), buffaloes (n = 30), goats (n = 15), and sheep (n = 10). A purposive sampling approach was adopted to target lactating animals with a history of reproductive disorders during the preceding two years. The selection criteria included a history of abortion, particularly during the last trimester of pregnancy, retained fetal membranes, repeated breeding, and the birth of weak or non-viable offspring. Information regarding the reproductive history of animals was obtained through consultations with livestock owners and local veterinary field assistants. This targeted sampling strategy was intended to investigate animals with a potentially increased likelihood of previous exposure to brucellosis.

Sample Collection and Transportation

Raw milk samples were collected aseptically from lactating cattle, buffaloes, goats, and sheep. Approximately 60 mL of milk was collected from each animal in sterile, properly labelled sampling containers. Before sample collection, the udder and teats were examined, and appropriate hygienic precautions were followed to minimize environmental and microbial contamination. Each sample was labelled with a unique identification number, animal species, sampling location, and relevant animal history. The collected samples were maintained under appropriate refrigerated conditions during transportation to the designated diagnostic laboratory and processed according to established laboratory biosafety and specimen-handling requirements.

Laboratory Detection and Identification of Brucella

Laboratory investigation was designed to identify *Brucella* in the collected raw milk samples. The diagnostic approach included appropriate screening and confirmatory identification methods, with particular attention to distinguishing *B. abortus*from other members of the genus *Brucella*. Bacterial isolation, where included in the approved study protocol, was restricted to appropriately equipped reference laboratory facilities operating under the required containment conditions. Suspected specimens and isolates were handled by trained personnel in accordance with institutional biosafety requirements because of the substantial risk of laboratory-acquired brucellosis.

The identification of suspected *Brucella* isolates was based on validated diagnostic and confirmatory procedures. Species-specific molecular identification was considered necessary to establish the presence of *B. abortus*, as detection of antibodies or nonspecific bacterial characteristics alone cannot conclusively establish species identity. Laboratory findings were recorded according to sample identification numbers to facilitate the calculation of the occurrence of the pathogen among the different livestock species and sampling locations.

Ethical Considerations

The study was conducted with consideration of animal welfare and ethical research principles. Permission was obtained from livestock owners before collecting milk samples from their animals. Sampling procedures were designed to minimize animal discomfort and avoid unnecessary handling or injury. The health and welfare of the animals were considered throughout the collection process. Appropriate

hygienic measures were adopted to prevent cross-contamination between animals and sampling equipment. All collected specimens were labelled and transported under suitable conditions to protect the integrity of the samples and prevent environmental contamination. Institutional ethical approval was obtained or sought in accordance with the requirements of the relevant university ethics committee.

Biosafety Measures

Because *Brucella abortus* is a highly infectious zoonotic pathogen with a recognized risk of aerosol transmission, appropriate biosafety precautions were considered essential throughout the investigation. Sample collection was performed by trained personnel using suitable personal protective equipment, including gloves, protective clothing, and other required protective measures. Potentially contaminated materials were handled and disposed of according to institutional biosafety procedures. Any confirmatory procedures involving viable *Brucella* organisms were restricted to appropriately equipped reference laboratories with trained personnel and the required containment facilities. These precautions were implemented to minimize occupational exposure and protect laboratory personnel, livestock handlers, and the surrounding environment.

Antimicrobial Susceptibility Assessment

The antimicrobial susceptibility component of the study was intended to investigate the antimicrobial response of confirmed *Brucella* isolates. However, susceptibility testing of *Brucella* requires validated laboratory procedures, appropriate reference standards, and specialized containment facilities because of the high risk of laboratory-acquired infection. Therefore, testing was considered only where scientifically justified and permitted by the relevant reference laboratory. The antimicrobial agents of interest included doxycycline, rifampicin, streptomycin, gentamicin, tetracycline, ciprofloxacin, and trimethoprim-sulfamethoxazole. Susceptibility findings were to be interpreted using validated species-specific criteria where available. Unvalidated Kirby–Bauer disc diffusion measurements were not considered sufficient for classifying *Brucella* isolates as susceptible, intermediate, or resistant.

Assessment of Human Exposure and Associated Risk Factors

The human exposure component was designed to investigate potential risk factors associated with brucellosis transmission among individuals living in the study area. Information regarding demographic characteristics, occupational activities, livestock ownership, animal handling practices, and consumption of raw milk and unpasteurized dairy products was considered. Particular attention was given to individuals involved in livestock farming, dairy production, animal slaughtering, and the handling of reproductive materials from livestock.

Potential exposure routes included direct contact with infected animals, contact with blood and reproductive discharges, consumption of contaminated raw milk or dairy products, and occupational exposure to potentially contaminated aerosols. Information regarding awareness of brucellosis, milk pasteurization practices, personal protective measures, and the management of potentially infected animals was also considered. The questionnaire-based assessment was intended to identify behavioural and occupational factors that might increase the likelihood of human exposure to *Brucella*.

Data Analysis

All collected information was entered into a structured database and analyzed using the Statistical Package for the Social Sciences (SPSS), version 25.0. Descriptive statistics, including frequencies and percentages, were used to summarize the distribution of milk samples according to animal species and sampling locations. The occurrence of *B. abortus*was calculated by dividing the number of confirmed positive samples by the total number of samples examined and multiplying the result by 100. Where appropriate, 95% confidence intervals were calculated to describe the precision of the estimated occurrence.

For the human exposure assessment, categorical variables, including occupation, educational status, raw milk consumption, livestock ownership, and direct contact with animals, were summarized using frequencies and percentages. Associations between potential exposure factors and the relevant human health outcome were evaluated using Pearson's chi-square test. A two-sided p-value of less than 0.05 was

considered statistically significant. The results were interpreted in relation to the cross-sectional nature of the study, and associations were not considered evidence of causation.

RESULTS

Isolation of Brucella abortus *from Raw Milk Samples*

A total of 80 raw milk samples were collected from cattle, buffaloes, goats, and sheep in Tehsil Paharpur, District Dera Ismail Khan. Initial bacteriological examination revealed suspected growth in 57 samples. Following subsequent examination, 48 samples were reported as positive for *Brucella abortus*, representing an overall isolation rate of 60.0%. The remaining 32 samples (40.0%) were negative.

The distribution of positive samples varied among livestock species. Buffaloes exhibited the highest isolation rate, with 22 positive samples out of 30 (73.33%), followed by goats, with 10 positive samples out of 15 (66.67%). In cattle, 13 out of 25 samples were positive (52.00%), whereas sheep demonstrated the lowest isolation rate, with 3 positive samples out of 10 (30.00%).

Table 1. Isolation rate of Brucella abortus from raw milk samples collected from different livestock species.

Animal species	Total samples examined (n)	Positive samples (n)	Negative samples (n)	Isolation rate (%)
Cattle	25	13	12	52.00
Buffaloes	30	22	8	73.33
Goats	15	10	5	66.67
Sheep	10	3	7	30.00
Total	**80**	**48**	**32**	**60.00**

The findings demonstrated that buffalo milk had the highest proportion of positive samples, followed by goat and cattle milk. Sheep milk showed the lowest proportion of positive samples. However, these differences describe the observed sample distribution and should not be interpreted as statistically significant differences in susceptibility without an appropriate statistical comparison.

Phenotypic and Serological Identification of Isolates

The suspected isolates were evaluated using morphological, staining, biochemical, and serological characteristics. Gram staining was reported to reveal faintly pink, Gram-negative short coccobacillary forms. Modified Ziehl–Neelsen (Stamp) staining also demonstrated weak acid-fast characteristics. These morphological and staining characteristics were considered consistent with presumptive identification of organisms belonging to the genus *Brucella*.

The biochemical profile reportedly showed positive oxidase, catalase, urease, and nitrate reduction reactions. Negative reactions were recorded for citrate utilization, indole production, Voges–Proskauer, and methyl red tests. Serological examination was reported to demonstrate agglutination with polyvalent anti-*Brucella* serum and anti-A monospecific serum, whereas no agglutination was reported with anti-M monospecific serum.

Table 2. Reported morphological, staining, biochemical, and serological characteristics of suspected Brucella isolates.

No.	Identification test or characteristic	Reported result
1	Gram staining	Gram-negative
2	Cellular morphology	Short coccobacillary forms
3	Modified Ziehl–Neelsen (Stamp) staining	Weak acid-fast reaction
4	Oxidase test	Positive
5	Catalase test	Positive
6	Urease test	Positive
7	Nitrate reduction test	Positive
8	Citrate utilization test	Negative

9	Indole production test	Negative
10	Voges–Proskauer test	Negative
11	Methyl red test	Negative
12	Polyvalent anti-*Brucella* serum	Positive agglutination
13	Anti-A monospecific serum	Positive agglutination
14	Anti-M monospecific serum	Negative agglutination

The reported morphological, biochemical, and serological findings were consistent with presumptive identification of *Brucella*. However, definitive species and biovar identification should be based on validated reference laboratory confirmation. The number of isolates subjected to each identification test must also be specified consistently in the final manuscript.

Antimicrobial Resistance Profile of the Isolates

The antimicrobial resistance results supplied for the bacterial isolates are summarized in Table 3. The highest reported resistance was observed against ampicillin (66.67%), followed by gentamicin (53.33%) and rifampicin (46.67%). Amoxicillin and ciprofloxacin demonstrated resistance rates of 45.00% and 40.00%, respectively. Streptomycin, tetracycline, and vancomycin each showed reported resistance rates of 33.33%, 30.00%, and 30.00%, respectively.

Table 3. Reported antimicrobial resistance profile of the bacterial isolates.

No.	Antimicrobial agent	Number tested (n)	Resistant isolates (n)	Resistance (%)
1	Streptomycin	15	5	33.33
2	Gentamicin	15	8	53.33
3	Ciprofloxacin	10	4	40.00
4	Tetracycline	10	3	30.00
5	Amoxicillin	20	9	45.00
6	Rifampicin	15	7	46.67
7	Ampicillin	15	10	66.67
8	Vancomycin	10	3	30.00

The results indicated variation in the reported resistance percentages among the tested antimicrobial agents. Ampicillin showed the highest proportion of resistant isolates, whereas vancomycin and tetracycline showed the lowest reported resistance percentages. These findings should be interpreted cautiously because validated antimicrobial susceptibility methods and organism-specific interpretive criteria are necessary for reliable susceptibility classification of *Brucella* isolates.

Potential Human Exposure Risk Factors Associated with Brucellosis

Several potential exposure pathways may contribute to human infection with *Brucella*, particularly among individuals involved in livestock management, dairy production, veterinary activities, and slaughterhouse operations. The major potential exposure pathways identified in this study are summarized in Table 4.

Table 4. Potential human exposure risk factors associated with animal brucellosis.

No.	Potential exposure risk factor	Possible route of transmission or exposure	Population potentially at risk
1	Consumption of raw or unpasteurized milk	Ingestion of contaminated dairy products	Consumers and dairy households
2	Direct contact with infected livestock	Contact with infected animals and their secretions	Farmers and livestock handlers
3	Handling aborted fetuses and placental materials	Contact with potentially contaminated reproductive tissues and fluids	Farmers and veterinary personnel

4	Assisting animals during abortion or parturition	Exposure to reproductive secretions and contaminated materials	Livestock attendants and veterinarians
5	Slaughterhouse activities	Contact with potentially infected animal tissues and fluids	Butchers and slaughterhouse workers
6	Inadequate use of personal protective equipment	Increased occupational exposure during animal handling	Veterinary and livestock workers
7	Improper disposal of animal waste	Contact with potentially contaminated animal waste and reproductive materials	Farmers and surrounding communities

Consumption of raw or unpasteurized milk represents an important potential foodborne route of brucellosis transmission. Occupational exposure may occur through direct contact with infected livestock, reproductive secretions, aborted fetuses, and placental tissues. Individuals involved in slaughtering, animal handling, and veterinary procedures may also be exposed, particularly when appropriate protective measures are not followed. Person-to-person transmission is rare and is not considered a major route of transmission.

The above factors represent potential human exposure pathways. In the absence of participant-level questionnaire data and human diagnostic testing, they cannot be interpreted as measured risk factors or as evidence of human brucellosis prevalence.

Table 5. Summary of the major findings according to the study objectives.

Study objective	Main findings
Objective 1: Isolation of *Brucella* from raw milk	48 of 80 samples were reported positive, giving an overall isolation rate of 60.00%.
Objective 2: Phenotypic and serological identification	Reported morphological, biochemical, and serological characteristics were consistent with presumptive identification of *Brucella*.
Objective 3: Antimicrobial resistance profile	The highest reported resistance was against ampicillin (66.67%), followed by gentamicin (53.33%).
Human exposure assessment	Raw milk consumption, direct livestock contact, handling reproductive materials, slaughterhouse activities, and inadequate protective measures were identified as potential exposure pathways.

Discussion

The present study investigated the occurrence of *Brucella* in raw milk samples collected from cattle, buffaloes, goats, and sheep with a history of reproductive disorders in Tehsil Paharpur, District Dera Ismail Khan. Of the 80 samples examined, 48 (60.0%) were reported as positive following the stated laboratory identification procedures. The findings indicate that raw milk from animals with a history of reproductive problems may represent a potential source of exposure to *Brucella* for farmers, animal handlers, milk vendors, and consumers. However, because animals were purposively selected on the basis of reproductive disorder history, the observed positivity should be interpreted as the proportion detected in this selected study population rather than the prevalence of brucellosis in all livestock in the district.

The positivity rate varied among animal species. Buffaloes showed the highest proportion, with 22 of 30 samples (73.33%), followed by goats, with 10 of 15 (66.67%), cattle, with 13 of 25 (52.00%), and sheep, with 3 of 10 (30.00%). These differences may reflect variation in the number of animals sampled, herd-level infection patterns, management practices, reproductive histories, and exposure to contaminated environments. Nevertheless, the sample sizes were relatively small, particularly for sheep, and the results should not be interpreted as evidence that one species is inherently more susceptible than another. Larger studies using representative sampling would be needed to establish reliable species-specific estimates.

The detection of *Brucella*-like organisms in raw milk is important from a public health perspective because milk can serve as a vehicle for transmission when consumed without adequate heat treatment. Infected animals may shed the organism in milk, reproductive discharges, and other materials associated with abortion and parturition. The risk may be increased in settings where raw milk is consumed, milk is handled without protective measures, or aborted materials are disposed of improperly. The World Health Organization (WHO, 2020) identifies consumption of contaminated, unpasteurized dairy products and direct contact with infected animals as important routes of human brucellosis transmission. Therefore, the findings support the need to improve milk hygiene, promote boiling or pasteurization, and strengthen awareness among livestock owners and milk consumers.

The association between reproductive disorders and brucellosis deserves particular attention. Abortion, retained fetal membranes, repeat breeding, and the delivery of weak or nonviable offspring may occur in animals infected with *Brucella*. However, these clinical signs are not specific to brucellosis and may also result from other infectious, nutritional, reproductive, or management-related conditions. Consequently, a history of reproductive problems should be considered an important reason for further investigation rather than sufficient evidence of infection. The inclusion of animals with such histories in the present study provided an opportunity to investigate a potentially higher-risk group, but it also limits the generalizability of the findings to the wider livestock population.

The reported laboratory findings should also be interpreted in light of the diagnostic methods used. Culture-based identification and conventional phenotypic characteristics can provide useful preliminary evidence; however, confirmation of *Brucella* species and biovars requires appropriate validated methods. Molecular confirmation, such as PCR targeting validated *Brucella*-specific markers, would strengthen the identification and help distinguish *B. abortus*from other *Brucella* species. Serological findings may support evidence of exposure but do not, on their own, demonstrate the presence of viable organisms in milk. Accordingly, the reported findings should be regarded as preliminary unless the isolates have been confirmed by a suitably equipped reference laboratory using validated identification procedures.

The findings can be considered alongside previous studies conducted in Pakistan. Khan et al. (2020) investigated bovine brucellosis using milk-based diagnostic approaches and reported evidence of infection in milk samples collected from different areas of Punjab. Nawaz et al. (2021) also reported evidence of *Brucella* exposure in buffalo and goat milk samples from the Islamabad Capital Territory using the milk ring test and milk indirect ELISA. Differences between those findings and the present study may be related to geographical location, livestock species, animal selection criteria, herd management, sample size, and diagnostic methods. In particular, serological detection and bacterial isolation measure different aspects of infection and should not be compared as if they were equivalent outcomes. The relatively high proportion observed in the present study may also be influenced by the deliberate selection of animals with a history of reproductive disorders.

Research conducted in Pakistan has further demonstrated the importance of laboratory-based investigation for identifying *Brucella* in livestock. Ali et al. (2014) described the isolation and characterization of bovine *Brucella* isolates using conventional and molecular approaches. Such investigations highlight the value of combining phenotypic identification with molecular confirmation to improve diagnostic confidence. More recently, Zeb et al. (2025) investigated antimicrobial-resistant *Brucella* in raw milk and animal faecal samples, drawing attention to the potential public health implications of contamination in livestock production environments. Although differences in study design and laboratory methods limit direct comparison, these studies collectively support the need for continued surveillance of brucellosis in animals and animal-derived food products in Pakistan.

The antimicrobial susceptibility findings reported in the present study require cautious interpretation. Susceptibility results for *Brucella* should be generated and interpreted using validated methods and appropriate organism-specific criteria. Routine antimicrobial susceptibility procedures and breakpoints developed for other bacterial species may not be suitable for *Brucella*. In addition, work involving viable *Brucella* isolates requires specialist laboratory facilities, trained personnel, and appropriate biosafety arrangements because of the organism's potential for laboratory-acquired infection. Therefore, antimicrobial susceptibility findings should not be used to recommend treatment unless the testing method, quality-control procedures, interpretive criteria, and isolate identification have been adequately

validated. Further confirmation by a reference laboratory would be valuable before drawing conclusions about antimicrobial resistance patterns.

From a One Health perspective, the findings highlight the interconnected nature of animal health, food safety, occupational exposure, and human health. Livestock owners, farm workers, veterinarians, milk collectors, and individuals involved in animal slaughter or the handling of reproductive materials may be exposed through direct contact with infected animals or contaminated materials. Consumers may also be exposed through the consumption of unboiled or unpasteurized milk. Preventive measures should therefore include regular screening of animals with reproductive problems, improved farm hygiene, safe disposal of aborted materials, use of protective clothing when handling potentially infectious materials, and public awareness campaigns promoting the consumption of boiled or pasteurized milk. Collaboration among veterinary, public health, and food safety authorities is also important for effective surveillance and control.

Several limitations should be considered when interpreting the results. First, the study was conducted in one tehsil and included a relatively small number of samples. Second, animals were purposively selected because of a history of reproductive disorders; therefore, the findings cannot be generalized to the entire livestock population of District Dera Ismail Khan or other regions. Third, the absence of a representative comparison group comprising animals without a history of reproductive disorders limits the ability to assess the relationship between reproductive history and *Brucella* positivity. Finally, further validated molecular characterization would improve confidence in species-level identification and provide more detailed information about the circulating strains. Future studies should include larger and more representative sample populations, relevant animal-level and farm-level risk factors, and confirmatory molecular testing.

Conclusion

The findings of the study provides preliminary evidence of *Brucella* positivity in raw milk samples collected from livestock with a history of reproductive disorders in Tehsil Paharpur. The findings draw attention to the potential role of raw milk in zoonotic transmission and emphasize the importance of improved animal health surveillance, hygienic milk production, safe handling of reproductive materials, and public awareness. Confirmatory laboratory testing and larger epidemiological investigations are recommended to establish the true distribution of *Brucella* infection and its associated risk factors in the study area.

REFERENCES

Ali, S., Ali, Q., Melzer, F., Khan, I., Akhter, S., Neubauer, H., & Jamal, S. M. (2014). Isolation and identification of bovine *Brucella* isolates from Pakistan by biochemical tests and PCR. Tropical Animal Health and Production, 46(1), 73–78. https://doi.org/10.1007/s11250-013-0448-6

Di Bonaventura, G., Angeletti, S., Ianni, A., Petitti, T., & Gherardi, G. (2021). Microbiological laboratory diagnosis of human brucellosis: An overview. Pathogens, 10(12), Article 1623. https://doi.org/10.3390/pathogens10121623

Jamil, T., Khan, A. U., Saqib, M., Hussain, M. H., Melzer, F., Rehman, A., Shabbir, M. Z., Khan, M. A., Ali, S., Shahzad, A., Khan, I., Iqbal, M., Ullah, Q., Ahmad, W., Mansoor, M. K., Neubauer, H., & Schwarz, S. (2021). Animal and human brucellosis in Pakistan. Frontiers in Public Health, 9, Article 660508. https://doi.org/10.3389/fpubh.2021.660508

Khan, A. U., Melzer, F., Hendam, A. H., Sayour, A. E., Khan, I., Elschner, M. C., Younus, M., Ehtisham-ul-Haque, S., Waheed, U., Farooq, M., Ali, S., Neubauer, H., & El-Adawy, H. (2020). Seroprevalence and molecular identification of *Brucella* spp. in bovines in Pakistan: Investigating association with risk factors using machine learning. Frontiers in Veterinary Science, 7, Article 594498. https://doi.org/10.3389/fvets.2020.594498

Maves, R. C., Castillo, R., Guillen, A., Espinosa, B., Meza, R., Espinoza, N., Núñez, G., Sánchez, L., Chacaltana, J., Cepeda, D., González, S., & Hall, E. R. (2011). Antimicrobial susceptibility of *Brucella melitensis* isolates in Peru. Antimicrobial Agents and Chemotherapy, 55(3), 1279–1281. https://doi.org/10.1128/AAC.00979-10

Nawaz, M., Khan, I., Shakeell, M., Yousaf, A., Naseer, Z., Munibullah, Zohaib, A., Hussain, R., & Ullah, Q. (2021). Bovine and caprine brucellosis detected by milk indirect ELISA and milk ring test in Islamabad Capital Territory, Pakistan. Pakistan Journal of Zoology, 53(1), 391–394. https://doi.org/10.17582/journal.pjz/20190906150953

Nawaz, Z., Shafique, M., Zahoor, M. A., Siddique, A. B., Ali, S., Arshad, R., Kausar, S., Khan, K., Asad, M., Rehman, A. U., & Masih, I. (2021). Sero-epidemiology and risk factor analysis of human brucellosis in Punjab, Pakistan: A cross-sectional study. Tropical Biomedicine, 38(3), 413–419. https://doi.org/10.47665/tb.38.3.084

Qureshi, K. A., Parvez, A., Fahmy, N. A., Abdel Hady, B. H., Kumar, S., Ganguly, A., Atiya, A., Elhassan, G. O., Alfadly, S. O., Parkkila, S., & Aspatwar, A. (2023). Brucellosis: Epidemiology, pathogenesis, diagnosis and treatment: A comprehensive review. Annals of Medicine, 55(2), Article 2295398. https://doi.org/10.1080/07853890.2023.2295398

Refai, M. (2002). Incidence and control of brucellosis in the Near East region. Veterinary Microbiology, 90(1–4), 81–110. https://doi.org/10.1016/S0378-1135(02)00248-1

Trott, D. J., Abraham, S., & Adler, B. (2018). Antimicrobial resistance in Leptospira, *Brucella*, and other rarely investigated veterinary and zoonotic pathogens. Microbiology Spectrum, 6(4). https://doi.org/10.1128/microbiolspec.ARBA-0029-2017

World Health Organization. (2020, July 29). Brucellosis. https://www.who.int/news-room/fact-sheets/detail/brucellosis

Zeb, S., Yasmin, H., Malik, I. R., Farah, M. A., Arya, V. M., & Hassan, M. N. (2025). Antimicrobial resistant *Brucella* spp. prevail in raw milk and animal feces of different livestock farms. BMC Microbiology, 25, Article 231. https://doi.org/10.1186/s12866-025-03930-8