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Seroepidemiology and Molecular Detection of Brucellosis among Pakistani Women with Spontaneous Abortions

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ABSTRACT

Background: Brucellosis is a major public health hazard, especially in tropical and subtropical regions. The disease poses a considerable hazard because of its quick transmission and the possibility of severe sequelae. **Objective**: This study aims to determine the seroepidemiology and molecular detection of brucellosis among Pakistani women with spontaneous abortion. **Methodology**: A cross-sectional study was conducted in different hospitals of Lahore over four months. A total of 165 abortion cases were investigated for brucellosis using a sequential diagnostic approach involving the Rose Bengal Plate Test (RBPT), Enzyme-Linked Immunosorbent Assay (ELISA), and Polymerase Chain Reaction (PCR). **Results**: Initial screening with RBPT identified 41 (24.84%) positive cases, while 124 (75.15%) were negative. The RBPT positive samples were further evaluated by ELISA, which confirmed 34 (82.9%) as positive and 7 (17.0%) as negative. Subsequently, PCR analysis of the 34 ELISA-positive samples revealed 27 (79.4%) were positive for Brucella DNA, whereas 7 (20.5%) were PCR-negative. **Conclusion:** These findings indicate a confirmed brucellosis prevalence of 16.36% (27/165) among the abortion cases, underscoring the importance of combining serological and molecular diagnostics for accurate detection of Brucella infection.

INTRODUCTION

A common and dangerous zoonotic illness that affects both humans and animals, brucellosis is brought on by the Brucella species, mainly B. melitensis and B. abortus. The disease, which was first discovered in Malta in 1860, is still common worldwide; in 2024, and more than 5 million cases were documented in more than 170 nations.[1,2] Those who work closely with livestock, such as farmers, veterinarians, and abattoir employees, are most vulnerable to transmission, which happens through direct contact with infected animals or the eating of unpasteurized animal products.[3,4] The infection causes nonspecific symptoms like fever, headache, and muscle discomfort, but it can progress to severe problems such as neurological and heart disorders.[5] Diagnosis relies on a combination of serological and molecular techniques, with PCR offering high sensitivity and specificity.[6,7] Despite its prevalence, limited data exist on the association between human brucellosis and spontaneous abortion in women, particularly in areas like Manga Mandi, Lahore. This study aims to investigate the seroepidemiology and molecular detection of brucellosis among spontaneously aborted women in this region to support disease control strategies and public health interventions.

MATERIAL AND METHODS

A cross-sectional study was conducted over four months in various hospitals across Lahore, A convenience sample method was used to choose 165 spontaneously aborted women. Data was acquired utilizing patient investigation forms and a semi-structured questionnaire. Blood samples (2–3 mL) were aseptically drawn, and serum was separated by centrifugation and stored at −4°C. Serological testing for anti-*Brucella* antibodies was performed using Rose Bengal Plate Test (RBPT) and ELISA (IgG, IgM) following standard protocols. DNA was extracted from clinical samples for molecular confirmation through PCR. Data were analyzed using SPSS software.

RESULTS

A total of 165 spontaneously aborted women from various hospitals, including Shamkay Bhattian, Social Security Hospital, Civil Hospital Manga Mandi, and other facilities,

were tested for brucellosis using RBPT, ELISA, and PCR. Overall, 41 samples (24.84%) tested positive, with Social Security Hospital reporting the highest number of negative cases (n = 34). The highest positivity was recorded in the 16–23 age group (17 out of 47), followed by the 24–31 group (11 cases), while fewer positive cases were found in older age groups (32–39: 8 cases; >40: 5 cases), indicating a higher prevalence among younger women as mentioned in Table 1.

Table 1

Sociodemographic Statistics of Patients

Hospital / Laboratory	Positive Cases	Negative Cases
Life Hospital (26)	10 (38.46) %	16 (61.53) %
Social Security Hospital, Lahore (41)	07 (17.0) %	34 (82.92) %
Civil Hospital Shamki Bhattian (39)	10 (25.6) %	29 (74.3) %
Civil Hospital Manga Mandi (31)	08 (25.8) %	23 (74.19) %
Other Hospitals and Laboratories (28)	06 (21.4) %	22 (78.5) %
Total (165)	41	124
Age Group (Years)		
16–23 (47)	17 (36.1) %	30 (63.8) %
24–31 (61)	11 (18.0) %	50 (81.9) %
32–39 (37)	08 (21.6) %	29 (78.3) %
>40 (20)	05 (25.0) %	15 (75.0) %
Total (165)	41	124

Most participants were from rural areas (72.7%) and over half were illiterate (54.5%). A majority had contact with animals (78.78%), mainly through occupational exposure (45.45%), with farming being the most common occupation (38.18%). Notably, over half had experienced two abortions (53.3%), indicating a possible link to brucellosis as mentioned in Table 2.

Table 2

Sociodemographic Statistics of patients

Parameters	Variables	Total Participants (n=165)	Percentage
Urbanicity	Urban	45	27.2%
	Rural	120	72.7%
Education	Illiterate	90	54.5%
	Primary	55	33.3%
	Secondary	20	12.12%
Occupation	Housewife	55	33.3%
	Farmer	63	38.18%
	Animal keeper	47	28.48%
Animal Contact	Yes	130	78.78%
	No	35	21.2%
Mode of contact	Direct	54	32.7%
	Indirect	36	21.8%
	Occupational	75	45.45%
Number Of abortions	01	45	27.2%
	02	88	53.3%
	>2	32	19.39%

Out of 165 abortion cases tested, 41 (24.84%) were RBPT-positive, 34 were ELISA-positive, and 27 were PCR-confirmed for active brucellosis. These findings highlight a significant association between *Brucella* infection and reproductive failure as mentioned in Table 3.

Table 3

Summary of Diagnostic Test Results and Abortion Cases (n=165)

Test Type	Result	Frequency	Percent (%)
Abortions	Total	165	100.0
RBPTs	Negative	124	75.15%
	Positive	41	24.84%
ELISA	Total	41	100.0%
	Negative	07	17.0%
	Positive	34	82.9%
PCR	Total	34	100.0%
	Negative	07	20.5%
	Positive	27	79.4%

DISCUSSION

Brucellosis is a globally prevalent zoonotic disease, with over six million cases and five million new infections reported annually across 170 countries. In Lahore, Pakistan, it is endemic, yet few studies have focused on its occurrence in women with spontaneous abortions. This study found 41 RBPT-positive cases out of 165, with ELISA confirming 34 and PCR detecting 27. A significant correlation was observed between RBPT and ELISA, aligning with Khan et al. (2021),[8] while poor concordance with PCR echoed findings by Sharma et al. (2020).[9] Younger age groups (16–31 years) showed higher positivity, consistent with Li et al. (2021)[10], and Ahmed et al. (2020), suggesting increased exposure and immune response.[11] Life Hospital and Civil Hospital Shamkay Bhattian reported the highest positivity, similar to institutional trends noted by Mehmood et al. (2022).[12]

CONCLUSION

This study suggests that brucellosis is a major public health concern in Lahore, particularly among pregnant women, due to a lack of information, intimate animal contact, and use of unpasteurized dairy products. The findings emphasize the importance of focused public health measures, routine screening of high-risk populations, and early detection in order to limit the disease's impact.

Future Recommendations

This study focuses on the diagnostic gap between fast tests (RBPT) and traditional methods such as ELISA and PCR, emphasizing the importance of improving rapid test accuracy. Future studies should investigate merging RBPT with biomarker-based or PCR technologies to improve community-level diagnosis.

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