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Abstract

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Keywords: Brucella, pregnant women, Abortion, Rose Bengal Test, Elisa

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Abstract

Pregnant women may experience spontaneous abortions due to Brucella species, often transmitted through raw milk, animal contact, or infected partners. This study investigated the seroprevalence of brucellosis and associated risk factors among pregnant women with a history of abortion in Bahawalpur, Pakistan. Conducted in three hospitals, 151 serum samples were screened using the Rose Bengal Agglutination Test, with confirmation via ELISA. Logistic regression and descriptive statistics revealed two positive cases (1.3%; 95% CI). Significant associations with abortion included age, pregnancy stage, trimester, livestock contact, raw milk consumption, male occupation, residence, and socioeconomic status (all $p < 0.05$). Findings highlight the importance of brucellosis screening in pregnant women with a history of miscarriages or exposure to animals. Public health measures, including household control strategies, education campaigns, and industry-wide eradication efforts, are recommended to mitigate human brucellosis.

Keywords: Brucella, pregnant women, Abortion, Rose Bengal Test, Elisa

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Contents

Introduction

The bacterium genus Brucella is the causative agent of the severe zoonotic disease brucellosis, which primarily affects cattle and can be transmitted to humans through direct contact with infected animals or by consuming contaminated animal products (Berhanu & Pal, 2020; Moreno, 2014)]. Especially in areas where cattle rearing is a big source of income, it challenges both public health and the economy (Kanu, 2024). Most patients infected with Brucella suffer nonspecific symptoms such as fever, weakness, and pains in the joints. Such patients are likely to be misdiagnosed or to delay diagnosis (Dean et al., 2012). Because it has been associated with bad reproduction outcomes in women such as infertility, stillbirths, and miscarriages, brucellosis is a significant cause of concern that needs

the medical fraternity's attention (Arenas-Gamboa et al., 2016).

Many people suffer from brucellosis in poor countries around the world, especially in places that do not have basic medical services or regular vaccination protocols for animals (Zhang et al., 2018). Brucellosis is prevalent in Pakistan, which is a predominantly agricultural country. This might be attributed to the huge population of cows there, unaware people of much about diseases, and not enough ways to control them (Bagheri Nejad et al., 2020). The fact that cattle and human health are intertwined underlines the critical need for immediate, targeted research in order to get an understanding of the disease's effects and the processes by which it is transmitted (Piracha et al., 2024).

The seroprevalence of brucellosis in women who have a history of abortion is worthy of particular attention because it has the chance of acting as an underreported cause of abortion (Tschopp et al., 2021). As much as there is this risky factor, there seems to be a lack of available information which finds its way from Pakistan, especially in regions like Bahawalpur where the majority of its people depend on pastoralism as a source of survival together with cattle as animal sources of food (Shahzad et al., 2017). Other past studies among neighboring countries link brucellosis and concern over reproductive health. This is the circumstance under which such deficits in regional monitoring and diagnostic practices still exist (Njeru et al., 2016; Bagheri Nejad et al., 2020).

This research fills an essential gap in evaluating the seroprevalence of brucellosis among women who have undergone abortions in different hospitals in Bahawalpur, Pakistan. The illumination of the prevalence of the disease in this vulnerable community empowers the study to raise awareness of brucellosis in the endemic regions.

Research Objective

The assessment of seroprevalence of Brucella among the patients of Bahawalpur having a history of abortion and risk factors for pregnant females.

Methodology

Study Design and Settings

This study was conducted for a full calendar year from January 2023 to December 2023 at three different hospitals in Bahawalpur, including Jubilee Female Hospital, Sir Sadiq Abbasi Hospital, and Bahawal Victoria Hospital.

Inclusion and Exclusion Criteria

Bahawal Victoria Hospital, Sir Sadiq Abbasi Hospital, and Jubilee Female Hospital. Female patients, with histories of abortion, who were admitted into the gynecological wards between ages 18-45 years are included in this study. Patients, who had previous treatment documented or histories of other infectious diseases like malaria or tuberculosis, are excluded from this study. Patients aged less than 18 years or more than 45 years and pregnant women who had never experienced an abortion were excluded from the study.

Sample Size and Collection

Blood samples were collected from 151 female patients with a history of abortion who were admitted to gynecology wards. The following hospitals provided samples: Sir Sadiq Abbasi Hospital (16), Jubilee Female Hospital (19), and Bahawal Victoria Hospital (116). Using syringes, 4 mL of blood was aseptically drawn and transferred into serum separator tubes. The serum was kept in Eppendorf tubes at -40°C after being separated by centrifugation at 6,000 rpm for five minutes. The Enzyme-Linked Immunosorbent Assay (ELISA) and the Rose Bengal analysis (RBT) were used to analyze blood samples. To check for agglutination, 30 μL of serum and an equivalent quantity of RBT antigen were combined on a white tile and shaken for 8 minutes. Visible agglutination was a sign of positive findings. Anti-Brucella antibodies were found using an IDvet ELISA kit. To create antigen-antibody complexes, serum samples were diluted and incubated after wells were coated with Brucella abortus lipopolysaccharide. The S/P% ratio was used to examine the results; S/P% $>$ 120% was seen as positive, 110% $<$ S/P% $<$ 120% was regarded as questionable, and S/P% $\leq$ 110% was regarded as negative.

Data Collection

Each patient's questionnaire was filled out. The questionnaire required information regarding their age, gender, place of residence, occupation, socio-economic status, direct or indirect contact with livestock, consumption of raw milk, and any current or past symptoms such as fever, headache, fatigue, generalized ache, and nausea. Additionally, respondents were asked if any other household member had experienced symptoms of brucellosis. Female participants were requested to provide information regarding their past pregnancies and history of abortion.

Statistical Analysis

IBM-SPSS version 23.0 was used to analyze the data; counts and percentages were provided for age group, history of abortions, contact with animals, male and female jobs, hospital, domicile, and socioeconomic position. To determine if these factors were associated with one or more abortion instances, the Pearson Chi-Square test was used. In both univariate and multivariate models, the odds ratio with 95% CI was provided. Binary logistic regression was also used to predict the additional risk of multiple abortions as compared to patients having just one abortion. P-values below 0.05 were regarded as statistically significant, and the study's findings were graphically presented using bar graphs.

Ethical Approval

Ethical approval for this study was obtained from the Ethics Review Board to ensure compliance with ethical standards, including informed consent from all participants, confidentiality of data, and adherence to ethical guidelines related to human research.

Results

The baseline characteristics of the 151 studied patients revealed that the majority were aged 25–30 years (52.3%), with a mean age of 28.1 years (±5.3). Half of the participants (50.3%) experienced one abortion, while 33.8% had two, and 15.9% had three or more (table 1). Livestock contact was reported by 58.9%, with 52.3% having direct contact. Most males were laborers (51%), while 90.7% of females were housewives. Rural residents constituted 54.3%, and 79.5% belonged to the lower socioeconomic class. The majority of patients (76.8%) were from Bahawal Victoria Hospital. The seroprevalence of brucellosis was low, with only 2% testing RBT positive and 1.3% confirmed by both RBT and ELISA, while 96.7% tested negative.

Table 1

Baseline Characteristics of Studied Patients (N=151)

Characteristics		Number of Patients (n)	Frequency (%)
Age Group	18 - 25 years	33	21.9
	25 - 30 years	79	52.3
	>30 years	39	25.8
	Mean (±SD)	28.1	±5.3
Abortions	One	76	50.3
	Two	51	33.8
	Three	16	10.6
	Four	6	4.0
	Five	2	1.3
Livestock Contact	Yes	89	58.9
	No	62	41.1
Contact with Livestock	Indirect	72	47.7
	Direct	79	52.3
Occupation (Male)	Labor	77	51.0
	Farmer	33	21.9
	Shopkeeper	18	11.9
	Teacher	10	6.6
	Other	13	8.6
Occupation (Female)	Housewife	137	90.7
	Worker	14	9.3
Residence	Rural	82	54.3
	Urban	69	45.7
Socioeconomic Status	Lower	120	79.5
	Middle	31	20.5
Hospital	BVH	116	76.8
	Jubilee	19	12.6
	Civil	16	10.6

Characteristics		Number of Patients (n)	Frequency (%)
Seroprevalence of Brucellosis	RBT Positive	3	2.0
	RBT and ELISA Positive	2	1.3
	Negative	146	96.7

The association of abortions with age, stage of pregnancy, and trimester revealed significant findings (table 2). Women aged 25–30 years constituted the majority in both groups, but those with more than one abortion had a higher proportion aged >30 years (38.7% vs. 13.2%, $p=0.001$). Abortions beyond the 4th stage of pregnancy were significantly more common among women with multiple abortions (77.3%) compared to those with only one (18.4%, $p<0.01$). Regarding trimester, the first trimester was the most common for both groups, though its prevalence was lower in women with multiple abortions (45.3% vs. 64.5%, $p=0.03$).

Table 2

Association of Abortion with Age, Stage of Pregnancy, and Trimester

Variables		Abortions				Chi-Square	p-value
		Only one (n=76)		More than one (n=75)			
		n	%	n	%		
Age Group	18 - 25 years	21	27.6	12	16.0	13.23	0.001*
	25 - 30 years	45	59.2	34	45.3		
	>30 years	10	13.2	29	38.7		
Stage of pregnancy	1st	23	30.3	0	0.0	58.53	<0.01*
	2nd	19	25.0	8	10.7		
	3rd	20	26.3	9	12.0		
	4th or above	14	18.4	58	77.3		
Trimester	1st	49	64.5	34	45.3	7.01	0.03*
	2nd	19	25.0	34	45.3		
	3rd	8	10.5	7	9.3		

The association of abortions with livestock contact and raw milk consumption showed significant differences (table 3). Women with multiple abortions were more likely to have livestock contact (82.7% vs. 35.5%, $p<0.01$), with direct contact being predominant (77.3% vs. 27.6%, $p<0.01$). Additionally, raw milk consumption was significantly higher among women with multiple abortions (64.0% vs. 23.7%, $p<0.01$). These findings highlight livestock exposure and raw milk consumption as key risk factors for multiple abortions.

Table 3

Association of Abortion with Livestock Contact and Raw Milk Consumption

Variables		Abortions				Chi-Square	p-value
		Only one (n=76)		More than one (n=75)			
		n	%	n	%		
Livestock contact	Yes	27	35.5	62	82.7	34.66	<0.01*
	No	49	64.5	13	17.3		
Contact with livestock	Indirect	55	72.4	17	22.7	37.38	<0.01*
	Direct	21	27.6	58	77.3		
Raw milk	Yes	18	23.7	48	64.0	24.93	<0.01*

consumption	No	58	76.3	27	36.0

The association of male and female occupation, residence, and socioeconomic status with abortions revealed notable patterns (table 4). Among males, laborers were significantly more common in the group with multiple abortions (61.3% vs. 40.8%, p=0.005). Rural residence was also more frequent in women with multiple abortions (66.7% vs. 42.1%, p=0.002). Socioeconomic status showed a significant difference, with a higher proportion of women from the lower class experiencing multiple abortions (88.0% vs. 71.1%, p=0.01). Female occupation and hospital distribution did not show significant associations with abortion frequency (p>0.05).

Table 4

Association of Male and Female occupation, Residence and Socioeconomic status with Abortion

Variables		Abortions				Chi-Square	p-value
		Only one (n=76)		More than one (n=75)			
Occupation Male	Labor	31	40.8	46	61.3	15.02	0.005*
	Farmer	17	22.4	16	21.3		
	Shopkeeper	12	15.8	6	8.0		
	Teacher	10	13.2	0	0.0		
	Other	6	7.9	7	9.3		
Occupation Female	Housewife	68	89.5	69	92.0	0.28	0.59
	Worker	8	10.5	6	8.0		
Residence	Rural	32	42.1	50	66.7	9.17	0.002*
	Urban	44	57.9	25	33.3		
Socioeconomic Status	Lower	54	71.1	66	88.0	6.64	0.01*
	Middle	22	28.9	9	12.0		
Hospital	BVH	61	80.3	55	73.3	1.02	0.59
	Jubilee	8	10.5	11	14.7		
	Civil	7	9.2	9	12.0		

The risk estimation for more than one abortion showed significant associations with several factors (table 5). In the univariate model, stage of pregnancy (OR: 2.26, 95% CI: 1.70–3.00), livestock contact (OR: 8.65, 95% CI: 4.04–18.5), direct contact with livestock (OR: 8.93, 95% CI: 4.27–18.6), rural residence (OR: 2.74, 95% CI: 1.41–5.32), and lower socioeconomic status (OR: 2.98, 95% CI: 1.27–7.02) were significant risk factors. The multivariate model further highlighted the stage of pregnancy (OR: 2.21, 95% CI: 1.43–3.43) and direct livestock contact (OR: 50.4, 95% CI: 8.68–292.0) as strongly significant, while rural residence showed a protective effect (OR: 0.28, 95% CI: 0.08–0.97). These findings suggest that direct livestock contact and stage of pregnancy are critical contributors to the risk of multiple abortions.

Table 5

Risk Estimation of More Than One Abortion with Studied Parameters

Risk Factors	Univariate Model	Multivariate Model
Age (years)	1.11* (1.03-1.19)	0.91 (0.80-1.04)
Stage of pregnancy	2.26* (1.70-3.00)	2.21* (1.43-3.43)
Trimester	1.50 (0.92-2.45)	2.01 (0.73-5.48)
Livestock contact: Yes	8.65* (4.04-18.5)	22.5* (4.27-118.0)
Livestock contact: No	Reference	Reference
Contact with livestock: Direct	8.93* (4.27-18.6)	50.4* (8.68-292.0)
Contact with livestock: Indirect	Reference	Reference
Raw milk consumption: Yes	5.72* (2.82-11.6)	2.62 (0.80-8.54)
Raw milk consumption: No	Reference	Reference

Risk Factors	Univariate Model	Multivariate Model
Occupation Male: Labour	1.27 (0.39-4.14)	4.27 (0.22-82.7)
Occupation Male: Farmer	0.80 (0.22-2.92)	7.64 (0.31-183.0)
Occupation Male: Shopkeeper	0.42 (0.09-1.85)	0.57 (0.06-5.02)
Occupation Male: Other	Reference	Reference
Occupation Female: Worker	0.73 (0.24-2.24)	0.28 (0.01-4.91)
Occupation Female: Housewife	Reference	Reference
Residence: Rural	2.74* (1.41-5.32)	0.28 (0.08-0.97)
Residence: Urban	Reference	Reference
Socioeconomic Status: Lower	2.98* (1.27-7.02)	0.13 (0.08-2.31)
Socioeconomic Status: Middle	Reference	Reference

Descriptive data of RBT and ELISA findings from a sample of 151 people are shown in Table 6. According to the data, 96.7% (n=146) tested negative for RBT, whilst 3.3% (n=5) tested positive. In terms of ELISA, 98.7% (n=149) were negative and 1.3% (n=2) were positive.

Table 6

Descriptive on RBT and ELSIA Results (n=151)

Results		Number of Patients (n)	Frequency (%)
RBT Result	Positive	5	3.3
	Negative	146	96.7
ELISA Result	Positive	2	1.3
	Negative	149	98.7

Discussion

The present study confirmed lower-than-reported seroprevalence rates for RBT and ELISA among individuals with an abortion history, coming in at 1.3%. One study found a seroprevalence rate of 6.2% in Iran (Stube et al., 2020), while another found rates as high as 14% among Pakistani women who had abortions in the past (Ejaz et al., 2024; Strube et al., 2020;). This research may reflect lower seroprevalence due to regional changes in animal husbandry techniques, enhanced public health measures, or variations in diagnostic methodologies. The decline in incidence is possibly due to increased awareness among the public, easier access to healthcare services, and more severe laws in the research domain. These findings create the requirement for targeted public health interventions and ongoing surveillance of brucellosis reservoirs in endemic areas.

Age: 28.1 ± 5.3 (22.3% were between 25 and 30 years old). It is a known fact that, within this cutive age group, issues related to brucellosis are more common (Ali et al., 2021). This is in agreement with past studies that reported younger women are in their reproductive prime and are more at risk in regard to livestock contact, which increases their probability of developing brucellosis-related problems such as abortion. Bosilkovski and the team found a trend a little similar, which they pointed out was that we actually need to concentrate on the screening of younger groups. Furthermore, it demonstrated that 33.8% of participants experienced two abortions, while 50.3% had one. This prevalence matches with the results of Ali et al. (2021) in Pakistan, who reported that women who have several abortions have a higher rate of brucellosis (Ali et al., 2016). Women who have a history of abortion should be screened and prevented from brucellosis infection since the incidence of spontaneous abortion is raised.

Livestock contact was reported by 58.9% of respondents, and those who had had several abortions reported the most direct contact with livestock (77.3%). This finding is in agreement with previous studies, like Gharekhani et al. (2021) and Ali et al. (2021), who have shown that contact with cattle is a major risk factor for brucellosis (Ali et al., 2021; Gharekhani et al., 2021). The high prevalence of livestock exposure in our study suggests that zoonotic diseases, in general, and brucellosis, in particular, may be an important factor in the recurrent pregnancy loss of these individuals. The shift from indirect to direct contact among aborters who have had multiple abortions illustrates the greater danger posed by more direct types of exposure. Raw milk consumption was also strongly associated with a history of many

abortions, as 64% of the participants who experienced repeated abortions acknowledged consuming unpasteurized milk compared to only 23.7% of the respondents who experienced a single abortion. To strengthen this finding, Majzobi et al. (2022) emphasized that pasteurized milk was significant in inhibiting brucellosis (Majzobi et al., 2022). Women who have undergone many abortions are said to consume more raw milk, which evidences the role played by food in disease causation.

When stratified by socioeconomic class, 79.5% of women who had many abortions were from lower-class families. This aligns with what Munyua et al. (2021) revealed, which is that brucellosis affects vulnerable groups more due to a higher risk of jobs and limited access to healthcare services. We observed a straightforward relationship between a lower socioeconomic class and a higher risk of brucellosis, which signifies the need for directed efforts toward addressing these inequalities.

Strengths and Limitations

The study deals with the seroepidemiology of brucellosis in women who had undertaken induced abortions in Bahawalpur, Pakistan. It shows a positive correlation between physical contact with cattle, consumption of raw milk, and some socio-demographic variables. The results are validated by a low seroprevalence rate of 1.3%, consistent with regional healthcare access and public health services, and further by the application of stringent diagnostic techniques, namely, RBT and ELISA. The sample size is very small and thus limits the generalizability of the results, which can be a cause for concern because animal exposure rates are high in the region. Self-reported exposures to animals and other risk factors, like the ingestion of unpasteurized milk, are susceptible to recall bias. Larger samples and longitudinal designs would be warranted in future studies to confirm this finding and explore other potential risk factors.

Conclusion

This study by researchers in Bahawalpur, Pakistan, tried to determine the prevalence of brucellosis among pregnant women who had already been aborted. Most people living in Bahawalpur are rural and have a close relationship with animals, consume raw milk, and therefore are vulnerable to brucellosis. Brucellosis is a big public health issue because it may cause harm to pregnant women, as indicated in this study. A patient who presents with features of brucellosis, including a history of abortion, should be screened for the infection by performing blood tests. The findings have established the importance of effective controls and prevention measures in curbing zoonotic brucellosis, especially among one of the world's most vulnerable groups.

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