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Short Communication

Seroprevalence of Brucellosis in Human, Cattle, and Buffalo Populations in Pakistan

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Abstract: A total of 1500 serum samples were collected from cattle, buffaloes, and humans. The Rose Bengal plate test (RBPT) and enzyme-linked immunosorbent assay (ELISA) detected Brucella antibodies in 56 (10.18%) and 44 (8%) of the cattle, respectively. In samples collected from buffaloes 9.38% and 6.92% were positive, whereas 14% and 11% of the samples from humans were found to be positive by RBPT and ELISA, respectively. Among village workers a higher prevalence of brucellosis was recorded in females than in males. Abattoir-associated personnel also had a higher incidence of brucellosis. Results of the present study revealed that RBPT and ELISA can be used efficiently for mass screening of Brucella antibodies in both humans and animals, and that people that worked with animals had a higher incidence of brucellosis, indicating that they might have been infected by contact with infected animals and might act as carriers.

Key Words: Prevalence, brucellosis, bovine, human, Pakistan

Bovine brucellosis, a disease of major economic and public health importance, is a worldwide problem (1). The disease is predominantly an occupational illness in farm and livestock workers, veterinarians, slaughterhouse employees, meat inspectors, and laboratory personnel (2). Individuals consuming dairy products in areas of endemic infection and those that handle animals and animal carcasses are at high risk of contracting brucellosis (3,4). A survey conducted in Pakistan found that 6.79% of humans were positive for *Brucella abortus* antibodies (5). In different areas of Pakistan the prevalence of *B. abortus* in bovines ranged between 3.25% (6) and 4.4% (7). A study conducted by Buchanan et al. (8) revealed that brucellosis is an abattoir-associated disease and slaughterhouse workers have the greatest risk of contracting the disease.

The prevalence of brucellosis has been reported to be between 3.2% (9) and 3.4% (10) in humans, and 6.47% in cattle and sheep in Turkey (9). Chen et al. (11) used RBPT and SAT for the detection of *B. abortus* antibodies in cows in the U.S., but these tests are not practical when performed on a large number of samples for various infectious diseases, including brucellosis. ELISA can be used as a diagnostic test for the screening of antibodies, as it is reported to have a sensitivity of 95%-100% (12). Güllüce and Leloğlu (13) used ELISA and the milk ring test (MRT) for the detection of *B. abortus* antibodies in dairy cows in Kars, Turkey. Abuharfeil and Abo-Shehada (14) recommended RBPT and ELISA for mass screening of brucellosis in sheep in Jordan. Ganesan and Anuradha (15) used RBPT and Dot-ELISA for mass screening of brucellosis in bovines in India.

In Pakistan most studies on brucellosis were conducted on organized government livestock and private livestock farms (16), and, to some extent, in humans. Little is known about the prevalence of *B. abortus* in Pakistan at abattoirs and among humans that work with livestock. The present study investigated the prevalence of *B. abortus* in cattle at abattoirs, farms, and villages, as well as in humans in direct contact with livestock.

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A total of 1500 serum samples were collected, including 500 cattle and buffalo samples from livestock farms and villages, 700 cattle and buffalo samples from abattoirs, and 300 human samples.

Rose Bengal Plate Test (RBPT)

RBPT antigen for *Brucella* was obtained from The Veterinary Research Institute, Lahore, Pakistan. Complete slide agglutination of RBPT antigen and tested serum was recorded as positive within 1 min, partial agglutination was doubtful, and no agglutination after 3-4 min was considered negative, according to Morgan et al. (17).

Indirect ELISA

An indirect ELISA was standardized and used to detect *Brucella* antibodies in serum samples following Burrels and Dawson (18). The samples were considered positive if their optical density was equal to or greater than the mean of 3 ± 0.12 based on ELISA at the wavelength of 450 nm.

The results of the present study indicated that the prevalence of brucellosis in humans, based on RBPT, was 12.5%, 14%, and 15% in males at farms, villages, and slaughterhouses, respectively, versus 10%, 10%, and 11%, respectively, based on ELISA ($P < 0.05$). RBPT results indicated that the prevalence of brucellosis was 10% and 16% among females in farms and villages, respectively, versus 10% and 14%, respectively, based on ELISA ($P < 0.05$) (Table 1). Sex-wise prevalence of brucellosis in cattle and buffalo at slaughterhouses, based on RBPT, was 12% and 10% in males ($P > 0.05$), and 9% and 9.2% in females ($P > 0.05$), respectively, whereas ELISA-based seroprevalence was 8% and 7.33% ($P > 0.05$), respectively, in males, and 7%, and 6.8%, respectively, in females ($P > 0.05$) (Table 2). At farms and villages RBPT determined that the prevalence in cattle and buffalo was 20% and 15% in males ($P > 0.05$), and 9.56% and 8.69% in females, respectively ($P > 0.05$), whereas ELISA detected *Brucella* antibodies in 10% and 10% ($P > 0.05$) of males, and 7.39% and 6.52% ($P > 0.05$) of females, respectively (Table 3). Among the 550 cattle samples, RBPT showed that 56 (10.18%) were positive and ELISA showed that 44 (8%) were positive. Among the 650 buffalo serum samples, 61 (9.38%) and 45 (6.92%) were positive according to RBPT and ELISA, respectively (Table 4). RBPT determined that 42 (14%) serum samples were positive for brucellosis in humans versus 33 (11%) by ELISA ($P > 0.05$) (Table 4). The overall results of the present study showed that RBPT detected more cases of brucellosis than *B. abortus*-specific ELISA. A higher prevalence rate was observed in cattle than in buffaloes. Females in villages and abattoir workers had higher rates of seropositivity for brucellosis.

Table 1. Seroprevalence of brucellosis in human populations.

Human Samples	No. of Serum Samples Tested		RBPT Positive Samples		ELISA Positive Samples	
	Male	Female	Male	Female	Male	Female
Farm Workers	80	20	10 (12.5%)	2 (10%)	8 (10%)	2 (10%)
Villagers	50	50	7 (14%)	8 (16%)	5 (10%)	7 (14%)
Slaughterhouse Workers	100	——-	15 (15%)	——-	11 (11%)	——-

Table 2. Sex-wise prevalence of brucellosis in cattle and buffalo at slaughterhouses.

Sex	Cattle			Buffalo		
	No. of Samples	RBPT Positive Samples	ELISA Samples Positive	No. of Samples	RBPT Positive Samples	ELISA Samples Positive
Male	100	12 (12%)	8 (8%)	150	15 (10%)	11 (7.33%)
Female	200	18 (9%)	14 (7%)	250	23 (9.2%)	17 (6.8%)
Total	300	30 (10%)	25 (8.33%)	400	38 (9.5%)	28 (7%)

Table 3. Sex-wise prevalence of brucellosis in cattle and buffalo at private farms and villages.

Sex	Cattle			Buffalo		
	No. of Samples	RBPT Positive Samples	ELISA Samples Positive	No. of Samples	RBPT Positive Samples	ELISA Samples Positive
Male	20	4 (20%)	2 (10%)	20	3 (15%)	2 (10%)
Female	230	22 (9.56)	17 (7.39%)	230	20 (8.69%)	15 (6.52%)
Total	250	26 (10.4%)	19 (7.6%)	250	23 (9.2%)	17 (6.8%)

Table 4. Screening of brucellosis by RBPT and ELISA.

Species	No. of Serum Samples Tested	RBPT Positive (%)	ELISA Positive (%)
Cattle	550	56 (10.18%)	44 (8%)
Buffalo	650	61 (9.38%)	45 (6.92%)
Humans	300	42 (14%)	33 (11%)

Diagnosis of brucellosis is based on clinical findings, serological tests, and bacteriological isolation and identification. Serological tests may reveal false positive results; therefore, blood and clinical samples suspected of brucellosis should be cultured for confirmatory diagnosis. Alternatively, serological tests are relatively easy to perform and provide a practical advantage in detecting the prevalence of *Brucella* infection. The purpose of the present study was the determination and comparative evaluation of the seroprevalence of *B. abortus* infection in serum samples obtained from cattle, buffalo, and humans based on RBPT and ELISA techniques (19). The seropositivity rate of brucellosis was higher in abattoir workers because these people had daily direct contact with a large number of animals (8).

The prevalence of brucellosis among humans in the present study, based on RBPT, was 14%, which is higher than the previously reported (5,9,10) prevalence rates of 10.7%, 3.2%, and 3.4%, respectively. The prevalence of brucellosis in villages was higher among females (16% based on RBPT and 14% based on ELISA) than among males (14% based on RBPT and 10% based on ELISA) because more females in rural areas were involved in milking, butter production, and the care and management of animals than men. These findings contradict Çetinkaya et al. (10).

Nasir et al. (16) recorded 14.70% and 18.53% brucellosis seroprevalence rates in cattle at government and private farms, respectively, whereas in the present study the seroprevalence rate was 10.4% (RBPT). This is because the present study included villages in which 2-4 animals per household were kept.

The present study found prevalence rates of brucellosis in cattle and buffalo at slaughterhouses of 10% and 9.5%, respectively, with RBPT, and 8.33% and 7%, respectively, with ELISA, which is higher than the 2.4% and 3.33% in cattle and buffalo, respectively, reported earlier by Ajmal et al. (20); however, they studied slaughterhouse and livestock farms collectively.

The results of the ELISA test used in the present study revealed that it was more sensitive and reliable than RBPT and therefore can be used for screening serum samples (12-14). Consequently, it can be concluded that screening animals for brucellosis in villages and slaughterhouses is necessary, and that further attempts should be made to control this disease. In our opinion, RBPT and ELISA can be used for the screening of brucellosis in animal and human populations, but ELISA is more sensitive. RBPT can be used for primary screening of brucellosis cases because cross reactivity is present in the RBPT antigen and confirmation must be made with a specific serological test, such as *B. abortus*-specific ELISA.

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