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Research Article

Seroprevalence of Brucellosis in cattle of Swat Valley Khyber Pakhtunkhwa Pakistan

Azhar Khan[1], Masood Rabbani[1], Farmanullah[2*], Ihsanullah Kakar[2], Sarfaraz Ali Fazlani[2], Asma Babar[2], Mohammad Salim[3], Momen Khan[4], and Sajjad Ahmad[2]

1. Department of Microbiology (AK) and University Diagnostic Lab (MR), University of Veterinary and Animal Sciences Lahore, Pakistan
2. Faculty of Veterinary and Animal Sciences, Lasbela University of Agriculture, Water and Marine Sciences, Uthal, Balochistan, Pakistan
3. Departments of Forestry and Wildlife Management, University of Haripur, Khyber Pakhtunkhwa, Pakistan
4. Livestock and Dairy Development Department, Khyber Pakhtunkhwa, Pakistan

**Corresponding author's email: farman_aup@yahoo.com; farman.vas@luawms.edu.pk*

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Abstract

Brucellosis has been an emerging, zoonotic disease that is associated with chronic debilitating infections in humans and reproductive failure in domestic animals. The aim of this study was to investigate the seroprevalence of brucellosis in samples collected from cattle in Swat valley based on the Rose Bengal precipitation test (RBPT), milk ring test (MRT), and indirect enzyme-linked immunosorbent assay (i-ELISA for both milk and serum). A total number 0f 310 samples were processed during the study which consists of 200 serum samples and 110 milk samples. The 200 serum samples were collected from male and female cattle (n=11and n=189).The RBPT and i-ELISA (serum) detected antibodies against *B.abortus* showed 1.587%, 2.12% in females through both tests respectively, and 0.0% in males on both techniques. The milk samples (n=110) detected *B.abortus antibodies* 0.9%, 1.82% through MRT and i-ELISA (milk) respectively. The comparative efficacy of MRT and i-ELISA (milk) was also analyzed statistically by z-test, the data revealed insignificant results (p= 0.561) also statistical analysis of RBPT and i-ELISA (serum) findings showed insignificant results (p= 0.703) therefore both results are higher than the significant level (0.05).

Keywords: brucellosis; ELISA; Milk ring test; Rose Bengal plate test; Sero-prevalence

Introduction

Brucellosis is an infectious zoonotic disease that is associated with chronic debilitating infections in humans and reproductive failure in domestic animals [1]. *Brucella abortus* infection in cattle named as Bang's disease" is often shortened to just "bangs". In cattle, this disease is also called contagious abortion and infectious abortion is a dominant feature of the disease in cattle is abortion [2].

Brucellosis causes abortions, infertility, retention of placenta, stillbirth, and calf loss in animals, and results in huge economic losses to dairy farmers [3]. Although the exact prevalence of brucellosis in cattle and buffalo in Pakistan is unknown, yet it has been reported to vary from 3.25 % to 4.4 % in different areas of Pakistan [4].

There is also an occupational risk to veterinarians, packing plant workers, farmers, and Ranchers who handle infected animals and aborted fetuses or placenta. Unpasteurized milk was considered the prime source of brucellosis; most humans contract the disease by coming in direct contact with aborted fetuses, after birth, and uterine discharges of diseased animals or with infected carcasses at slaughter [5]. The Khyber Pakhtunkhwa is in the developmental stages in the livestock sector. Small farmers own almost 90% of the Khyber Pukhtoonkhwa's livestock and are landless, providing an opportunity for improving the quality of their lives. There is a dire need of screening these animals for this important zoonotic disease. Different assays can be used for screening mass-scale populations that includes SAT, Milk Ring Test (MRT), RBPT, and ELISA. ELISA may be used as a diagnostic test for the screening of antibodies, as it is reported to have a sensitivity of 95%-100% [6]. The RBPT and Dot-ELISA were used for mass screening of brucellosis in bovines in India [7]. Furthermore, [8] has recommended RBPT and ELISA for mass screening of brucellosis in Jordan. In Pakistan, most studies on brucellosis are conducted on organized government livestock and private livestock farms [9], involving, to some extent, humans. A little is known about the prevalence of *B. abortus* in Khyber Pukhtoonkhwa particularly in Swat Valley. The aim of this study was to look into the prevalence of *B. abortus* in cattle and to compare the efficacy of RBPT. MRT and I-ELISA for detection of antibodies against *B. abortus* were also studied.

Materials and Methods

A total number 0f 310 cattle samples were processed during the study which consists of 200 serum samples (11 male and 189 female) and 110 milk samples. All samples were subjected separately to serological tests and MRT for the detection of any positive case of brucellosis in target animals. *Rose bengal plate test (RBPT)* The test was carried out using Bengatest kit (Synbiotics, USA) according to the manufacturer's instruction. Briefly, 30 ul of test sera were added on a clean glass slide followed by the addition of the same quantity of rose Bengal antigen. Bothe antigen and antiserum were mixed together. Agglutination of RBPT antigen and test serum was recorded as positive within 4 minutes, partial agglutination as doubtful, and no agglutination as negative.

Indirect ELISA (serum) an indirect ELISA kit (Svanova, Sweden) was used to detect Brucella antibodies in serum samples following the manufacturer's instruction. Briefly, 100 ul (1:50 in dilution buffer) serum sample, positive and negative control sera were added in the respective well pre-coated with *B. abortus* antigen followed by incubation at 37°C for an hour. The wells were rinsed thrice using 100 ul (1:20 in distilled water) PBS-Tween buffer followed by the addition of 100 ul HRP labeled conjugate and incubation at 37°C for an hour. Upon washing wells thrice again, 100 ul of substrate solution was added to all the wells followed by incubation at room temperature for 15 minutes. Finally, 50 ul of stop solution was added to each well and optical density was measured at 405 nm using an ELISA reader (Thermo Electron, Finland). The PP (Percent Positivity) was measured according to the formula: Test sample OD PP (Percent Positivity) = 100 Positive control OD The samples were considered positive if their PP was equal to or greater than 25 and negative if

less than 25. *Indirect ELISA (milk)* the milk samples were also processed using the same procedure as mentioned above except that undiluted 100µl each of the milk sample was used instead of diluting samples as in the case of serum samples.

Milk Ring Test: The fresh milk samples (110) collected from villages was tested for brucellosis using MRT antigen procured from Veterinary Research Institute (VRI), Lahore. One drop (0.03ml) of stained brucella antigen was added to 01ml of whole milk previously kept at 4^{o}C under overnight refrigeration. The test results were studied after incubation for 1 hour at 37 ^{o}C. A positive reaction was indicated by a stained cream layer over the white column of milk

Results

The study revealed that the prevalence 1.5% in cattle (n=200). The sex-wise prevalence in cattle was also studied. In males, the prevalence was 0.0% (n=11) but it was 1.587% in females (n=189) through RBPT. All the serum samples were also processed through i-ELISA to compare the prevalence %age with that of the *Rose bengal plate test*. The study also revealed that the prevalence through i-ELISA in cattle was 2 % (n=200). The sex-wise prevalence in target animals was also studied through indirect ELISA. In cattle males, the prevalence was 0.0% (n=11) but it was 2.12% (n=189) in female cattle. The milk samples from Cattle were also subjected to Milk Ring Test and i-ELISA depicted the prevalence of 0.9%.and1.82% (n=110) on both test respectively. The *Rose bengal plate test* and i-ELISA tests were compared statistically by z-test, showed insignificant results (p= 0.703) which is higher than the significant level. (0.05) shown in (Table 1).

The comparative analysis of MRT and i-ELISA (milk) was also done statistically by z-test, revealed insignificant results (p= 0.561) which are higher than the level of significance (0.05) shown in (Table 2).

Table 1. RBPT and I-ELISA (serum) results in cattle

Sex	Samples tested	RBT%age		*i-ELISA %age*	
		+ ve	-ve	+ ve	*-ve*
Male	11	0 (0%)	11 (100%)	0 (0%)	*11 (100%)*
Female	189	3 (1.587%)	186 (98.4%)	4 (2.11%)	*185 (97.88%)*
Total	*200*	*1.5%*	*98.5%*	*2%*	*98%*

Table 2. MRT and I-ELISA (milk) results in cattle

sex	Samples tested	MRT %age		*I-ELISA (milk)*	
		+ve	-ve	+ve	*-ve*
female	110	1	109	2	*108*
Total	*110*	*(0.9%)*	*(99.1%)*	*1.82%*	*98.2%*

Discussion

Serodiagnosis of brucellosis is usually based on a history of abortion in the last trimester along with the retained placenta, clinical findings, several serological tests, bacteriological isolation, and identification. As compared to bacterial culturing, serological tests are relatively easy to perform and provide a more a practical advantage in detecting the antibodies against *B. abortus*. Among the various serological test, RBPT and ELISA are commonly used for screening

the infection against Brucella [8]. All the serum samples were tested through RBPT and i-ELISA while milk samples were processed through MRT and i-ELISA. This study showed a greater incidence of Brucellosis in female cows (1.587%) than male cattle (0.0%). These findings are again similar to the results of [10-12] who on the basis of their research work concluded that the incidence of the disease amongst female animals was higher than that of male animals. Same findings were observed through i-ELISA in female cattle (high prevalence in females 2.12% than males-0.0%). The higher incidence in female animals might be due to stress during pregnancy and lactation period and epidemiology of the disease, predilection site of the female reproductive organs, placenta, and fetus for the causative agent. The comparison of three serological techniques i.e. (RBPT, MRT with i-ELISA) revealed that out of 200 serums and 110 milk samples 03 and 04 samples were positive through RBPT and i-ELISA respectively. Furthermore, 02 milk samples were found positives by milk i-ELISA instead of only 01 positives by MRT indicating slightly high sensitivity and specificity of i-ELISA. These results are in close agreement with the findings of [12], who stated that ELISA detects more sensitivity as compared to RBPT and MRT. In the present study, it is obvious that I ELISA a more sensitive than RBPT, as it detected of positive cases. These lines are in parallel to the observation of [12-17] that ELISA would be more specific than RBPT and useful for epidemiological surveillance for brucellosis. Elisa is the most sensitive test therefore it should be preferred and elimination of reactors. Elimination of the reactors means to eradicate disease. The other test i.e. RBPT and MRT is just for screening.

Authors' contributions

Conceived and designed the experiments: A Khan & M Rabbani, Performed the experiments: A Khan, FarmanUllah & M Salim, Analyzed the data: A Khan, SA Fazlani, A Babar & S Ahmad, Contributed materials/ analysis/ tools: A Khan, M Khan, Farmanullah & S Ahmad, Wrote the paper: A Khan & M Rabbani, Revised the paper: I Kakar, Farmanullah, & M Salim.

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