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SEROPREVALENCE OF BOVINE BRUCELLOSIS USING INDIRECT ELISA IN QUETTA BALOCHISTAN, PAKISTAN

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ABSTRACT

Brucellosis is considered the most important zoonosis worldwide with high prevalence among livestock. A total of 780 cattle (n = 405) and buffalo (n = 375) serum samples were collected from abattoir Quetta, Balochistan and evaluated for the presence of antibrucella antibodies, using Rose Bengal plate test (RBPT) and indirect enzyme-linked immunosorbent assay (i-ELISA) respectively. The overall prevalence of 3 % and 3.20 % was recorded through RBPT and i-ELISA respectively. In samples collected from cattle (n=405) the higher seroprevalence of 3.95 % and 5.9 % was recorded than buffaloes (n= 375) with 2.1% and 0.265 using RBPT and i-ELISA respectively. Similarly a relatively higher seroprevalence of was found in female animals as 3.72 % and 3.88 % using RBPT and ELISA respectively. While the prevalence in male was 0.6% using both the test. These results revealed that RBPT and ELISA can be used for large scale screening of brucella antibodes in animals. These findings suggest an alarming situation of bovine brucellosis in animals slaughtered at Quetta abattoir in Balochistan.

Key Words: Brucellosis, Buffalo, Milk Ring Test, indirect enzyme linked immunosorbent assay.

INTRODUCTION

Brucellosis remains an economically important highly contagious and zoonotic bacterial disease of animals worldwide (OIE, 2000). It persists in regions where infection in animals, especially men and livestock, has not been brought under control and where, consequently, transmission of the infection to humans frequently occurs. It is re-emerging infectious disease which is closely associated with the evolution of mankind as an agrarian society linked to the practice of shepherding and popularization of animal husbandry (Basappa, 2008). The disease causes significant economic losses including abortion, loss in milk production, low fertility rates and cost of replacement of animals (McDemott and Arimi, 2002). It is a global problem of wild and domestic animals, especially cattle, sheep and goats causing a decrease in reproductive efficiency and an increase in abortion rate (Rijpens *et al.,* 1996). It is a significant public health problem in an agricultural country like Pakistan, where the vast majority of the population is involved in land cultivation and livestock farming. Although the exact prevalence of the brucellosis in bovine is unknown in Pakistan; but has been reported to vary from 3.25% to 4.4% in different areas of Pakistan (Naeem *et al.,* 1990). In dairy animals major economic losses due to brucellosis include loss of calves and reduced milk yield in females and infertility in males (Saeed *et al,* 1968). A high prevalence of brucellosis is reported in Faisalabad area of Pakistan by Iftikhar *et al.* (2008) as 10.18% and 8% in cattle using RBPT and ELISA respectively.

As most of the geographical land of the Balochistan province is mountainous and 70% population is scattered as rural and most of the people practice nomadic life. The present study has been carried out to estimate the seroprevalence of bovine brucellosis RBPT and i- ELISA.

MATERIALS AND METHODS

The study comprised of 780 randomly collected serum samples from cattle (n = 405) and buffalo (n = 375) respectively in abattoir of Quetta city, Balochistan Pakistan. The samples were collected both from cattle (Male = 155 and Female = 250) and buffalo (Male = 7 and Female = 368) respectively to observe seroprevalence of antibodies against *B. abortus*. The blood samples were collected aseptically from each animal, serum was separated in a sterile prelabelled microfuge tube (1.5 ml). Maximum possible hygienic measures were adopted during collection, transportation and processing of these samples. All the serum samples were subjected to RBPT and i-ELISA

Rose Bengal Plate Test (RBPT): The test was carried out by following the method as described by Morgan *et al*. (1978) using the antigen procured from Veterinary Research Institute, Lahore. A drop of serum sample and a drop of Rose Bengal antigen were added onto a clear

glass slide. The contents of both drops were mixed thoroughly and gently. The reaction was observed after few minutes. Complete agglutination was recorded as positive, partial as doubtful and no agglutination as negative.

Indirect Enzyme Linked Immunosorbent Assay (i-ELISA): The indirect ELISA (i-ELISA) kit for the detection of antibrucella antibodies was obtained from M/S Svanova, Sweden and the testing procedure was followed as per manufacturer's procedure. The sample dilution buffer (100µl) was added to each well along with positive and negative control to the selected precoated antigen wells, respectively. The plate was shaken thoroughly, sealed and incubated at 37 ^{o}C for one hour. The plate was rinsed 3 times with PBS- Tween buffer followed by addition of 100µl of Horse reddish peroxidase (HRP) conjugate (prepared in 11.5 ml PBS Tween buffer diluted in distilled water) to each well and incubated at 37 ^{o}C for one hour. The plates were again rinsed thrice, and 100µl substrate solutions (tetra methyl benzidine) was added to all wells. Micro titration plate was incubated for 10-15 minutes at room temperature. Finally 50 µl of stopping solution was added to each well for stopping the reaction (Macmillan, 1990). The optical densities (OD) of the control and test sample wells were adjusted at 405 nm using an ELISA reader (Thermo Electron, Finland). The results were calculated in terms of PP (Percentage Positivity) value and interpreted accordingly. The serum samples showing PP value lesser than 25, were considered negative and those showing PP value equal or greater than 25, were taken as positive.

RESULTS AND DISCUSSION

The overall seroprevalence of antibodies against *B. abortus* was found in cattle and buffalo as 3% and 3.2% using RBPT and i-ELISA respectively (Table-1). The results indicated a comparatively lower prevalence of brucellosis in cattle than buffalo as supported by previously reported 3.97 % prevalence using Rose Bengal Plate Test (RBPT) and Serum agglutination Test (SAT) by (Faqir, 1991). Similarly a much higher prevalence of 8.5 % was recorded in cattle and buffaloes in Quetta (Shafee *et al*., 2011).

The data collected also revealed that prevalence of bovine brucellosis is much higher in cattle than in buffaloes. It was 3.95 % and 5.9 % in cattle and 2.1 % and 0.26 % in buffaloes using RBPT and i-ELISA, respectively. This may be due to the fact that buffaloes are naturally resistant to brucellosis. Borrielo *et al.* (2006) have described buffaloes naturally resistant to *B. abortus* determining a correlation between the BB genotype and resistance to *B. abortus* infection. Further, these findings are in agreement with Abbas *et al.,* (2009) and Iftikhar *et al. (*2008) who reported higher prevalence of brucellosis in cattle (10.5%) than (1.9%).

The sex wise prevalence of brucellosis was also recorded in both species using RBPT and i-ELISA. In male animals 0.6 % prevalence was observed using both the techniques. In female animals 3.72 % and 3.88 % prevalence was recorded using RBPT and ELISA respectively. These findings reflect much higher prevalence in female than male animals. These findings are in agreement with the findings of Nuru (1975); Ahmad *et al*. (1995), whereas disagree with Qureshi and Bhatti, (1968) who reported high prevalence of brucellosis in male than female animals. However, most of recent studies have suggested that brucellosis has nothing to do with sex but is more in old aged animals.

Table 1: Seroprevalence of brucellosis in Cattle and buffalo in Quetta abattoir using *RBPT and **i-ELISA

Species	No. of animals	Positive by *RBPT (%)		Positive by**i-ELISA (%)		Overall Seroprevalence	
		Male	Female	Male	Female	*RBPT	**i-ELISA
Cattle	**405**	1/155 (0.6 %)	15/250 6 %	1/155 (0.6 %)	23/250 (9.2 %)	16/405 (3.95%)	24/405 (5.9 %)
Buffalo	**375**	00/7 (0%)	08/368 (2.19 %)	0/7 (00%)	1/368 0.27%	08/375 (2.1%)	01/375 (0.26%)
Total	**780**	1/162 **(0.6 %)**	23/618 **(3.72 %)**	01/162 **(0.6 %)**	24/618 **(3.88 %)**	24/780 **(3.00 %)**	25/780 **(3.20 %)**

*RBPT= Rose Bengal plate Test.
**i-ELISA= indirect Enzyme linked immunosorbent assay.

In the present study the ELISA has shown more sensitivity to detect antibodies against *B. abortus* as compared to RBPT. This finding is in accordance with the findings of Kerkhofs *et al.* (1990), Vanzini *et al.* (2001), Chakra *et al*. (2000), Iftikhar *et al.* (2008) and Kang, (2000) who also reported ELISA as more sensitive than other conventional assays such as RBPT and MRT. Consequently, it can be therefore concluded that regular screening of animals for brucellosis in villages or areas where there is more frequent contact between human and animal population such as slaughterhouses and farms is

necessary and further attempts should be made to control this disease.

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