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RESEARCH ARTICLE

Seroprevalence of Ovine Brucellosis by Modified Rose Bengal Test and ELISA in Southern Punjab, Pakistan

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ABSTRACT

Pertaining to reported high prevalence of brucellosis in Pakistan, district Layyah on account of having thick sheep population was selected for this study. A total of 384 sheep blood samples were collected randomly from different selected private herds in the district, and tested through Modified Rose Bengal (mRB) test and Indirect Enzyme Linked Immunosorbent Assay (iELISA) for the serological analysis against the *Brucella* antibodies. Positive samples from these two tests were further subjected to Competitive Enzyme Linked Immunosorbent Assay (cELISA). The individual based seroprevalence of brucellosis in sheep was found to be 7.0% by mRB. Herd based prevalence was 42.5%. The highest ($P<0.05$) seroprevalence (16.8%) was observed in Tehsil Layyah as compared to Tehsils Karor (2.3%) and Choubara (4.5%). Non-significant differences were recorded between breeds, age and sex groups and also for the animals with or without history of abortion. In case of indirect and competitive ELISA, no sheep serum sample was found to be positive.

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INTRODUCTION

Brucellosis is known as the second most zoonotic problem in the world (Cutler and Whatmore, 2003). It persists as a problem for animals all over the world (Bricker, 2002) and results in considerable economic losses due to abortion, decreased fertility rates, less milk production and cost of animal replacements (McDermott and Arimi, 2002). High prevalence of brucellosis has been reported in subcontinent especially in Pakistan and India. In Pakistan, brucellosis is an ignored disease and no official strategy for brucellosis control and eradication exists. Consequently, no necessary actions have been taken on to restrict the spread of the disease in various private and government herds (Abubakar *et al.,* 2011).

Brucella melitensis is responsible for ovine brucellosis (Garin-Bastuji *et al.,* 1998). The main symptoms of the infection in sheep include reproductive problems such as abortion, placenta retention, stillbirth, birth of feeble offspring. Usually abortion occurs only once in the animal life. Inflammation of testis, seminal vesicle, epididymis and ampulla has been reported in rams with low quality semen production resulting in infertility (Megid *et al*., 2010).

Isolation and identification of *Brucella* spp. is regarded as the definite diagnosis of the disease. As this process is time consuming, hazardous and faces the disadvantage of being unpractical to be applied at national scale in control strategies so serological tests are mostly preferred (Ferreira *et al*., 2003). There are a number of serological tests which have been used for screening or confirmatory diagnosis of brucellosis (Elsheikh *et al*., 2012). Mostly commonly used Rose Bengal Plate test (RBPT) and complement fixation test (CFT) combination has enjoyed globally sporadic success in eradication of brucellosis from cattle (Ferreira *et al*., 2003). Nonetheless, this combination is advocated to be less effective in case of small ruminants (Ferreira *et al*., 2003). A mRB test protocol considerably boosts up the RB antigen sensitivity without disturbing its specificity (Ferreira *et al*., 2003). The mRB test and iELISA have higher sensitivity than any other test for *Brucella* diagnosis and can usefully replace the current RBPT procedure used for screening purpose (Ferreira *et al*., 2003).

District Layyah has thick population of sheep with large number of small private herds as most of the rural families are dependent upon small sheep herds for their earnings. Keeping in view the importance of sheep for small farmers, present study was planned to check the

seroprevalence of brucellosis in sheep and to suggest the measures to minimize the problem for small holders.

MATERIALS AND METHODS

The study was carried out in District Layyah, comprising of three tehsils i.e., Layyah, Choubara and Karor Lal Esan, located in Southern Punjab of Pakistan at geographic coordinates of 30°58′ North latitude and 70°56′ East longitude with an altitude of 143 meters. Hot climate with a maximum temperature of 53ºC and low rainfall characterize the district. Choubara Tehsil consists of forests and is nearly barren while the other two tehsils, being near to river Indus, are developed agriculturally. This study was based upon unknown prevalence of brucellosis in sheep in the study area. For calculation of sample size from an unknown size of population, relevant formula used for a 95% confidence interval is given as:

$$n = \frac{1.96^2 P_{exp}(1-P_{exp})}{d^2}$$ (Thrusfield, 2007)

With 50% expected prevalence and 5% desired absolute precision; we had following number of samples:

$$n = \frac{1.96^2\ 0.50\ (1-0.50)}{0.05^2}$$

$$n = 384$$

Accordingly, a total of 384 blood samples were randomly collected randomly from different private herds, without any previous record of vaccination against *Brucella* species. During blood sampling, information regarding the animals was collected by using a structured questionnaire. Blood samples without anticoagulant were collected and kept overnight in slanting position at room temperature and then centrifuged at 1000 rpm for 5 minutes to allow proper serum separation. Serum was stored at -20ºC till its use for serological analysis.

Two serological tests, i.e., mRB test and iELISA (Svanovir Biotech AB, Uppsala, Sweden) were performed for the analysis of all the serum samples and then positive samples resulted from the two tests were confirmed by cELISA (COMPELISA-Veterinary Laboratories Agency, UK). Both the ELISA was performed according to the instructions of the manufacturer. Control positive and control negative serum samples were procured from Onderstpoor Veterinary Research Institute, Onderstpoor, South Africa.

Antigen of *Brucella abortus* for mRB test was obtained from Veterinary Research Institute, Lahore. The test was performed following the procedure described by Ferreira *et al.* (2003). iELISA and cELISA were performed by kit method following the manufacturer's instructions (Svanova Biotech AB, Uppsala, Sweden). The data on different variables, obtained from the study, were analyzed statistically using Chi-square test (Steel *et al.*, 1997).

RESULTS

The overall prevalence of the disease was 7.0% (27/384) by mRB while herd based seroprevalence in the district came out to be 42.5%. The seroprevalence of the disease was much higher (P<0.0001) in Tehsil Layyah as compared to other two tehsils i.e., Karor and Choubara (Table 1). Seroprevalence rate showed no affinity for sex (P<0.05). In case of age, the seroprevalence of brucellosis increased with increasing age as it was 3.3, 8.2 and 8% in 1-2.5 years, 2.5-4 years and 4 years to above age groups with non-significant difference. Regarding the association between seropositivity against *Brucella* with abortion history, 14.2% of the test population with abortion history was found seropositive and this percentage was 6.9 for samples without any history of abortion, anyhow this difference was not significant statistically (P□0.05). No sheep serum sample was found positive with indirect and competitive ELISA.

Table 1: Sero-prevalence of ovine brucellosis by mRB test

Parameters		Total	Positive	%	Chi-square value	P-value
Tehsil	Layyah	101	17	16.8	20.6428	0.0001*
	Karor	128	03	2.3		
	Choubara	155	07	4.5		
Sex	Male	368	26	7.1	0.0001	0.9911
	Female	14	01	7.1		
Age(years)	1-2.5	90	03	3.3	2.4653	0.2915
	2.5-4	194	16	8.2		
	4-above	100	08	8.0		
Breed	Kajli	137	11	8.0	0.3245	0.5689
	Thali	247	16	6.5		
Abortion	Abortive	377	26	6.9	0.5740	0.4487
History	Non-abortive	07	01	14.3		

DISCUSSION

The prevalence of brucellosis is increasing in Pakistan especially in large sized dairy herds. No doubt, different serological tests have been used in various studies to find out the incidence of the disease in the country (Abubakar *et al.*, 2012), but all these studies employed comparatively less reliable tests like SAT and RBPT. The main objective of the present study was to peep into much seroprevalence of brucellosis using diagnostically more reliable iELISA and cELISA (Teshale *et al*., 2006).

The seroprevalence of sheep brucellosis revealed from this study to be much lower than reported by Hamidullah *et al.* (2009), who found 34.8% sheep positive to *Brucella* in Kohat. One possible reason for this lower seroprevalence in the present study could be that the study involved small house hold herds having less number of animals than number of animals at the farms focused in previous study by Hamidullah *et al.* (2009). As, small sized herds are less likely to have at least one seropositive animal than large sized herds and crowdedness in larger herds may positively affect the seropositivity rate (Al-Majali, 2005).

The study results were comparable to Negash *et al*. (2012) who reported the prevalence of brucellosis in ovine to be 8.7% in Ethiopia. The current prevalence rate is also higher when compared to (1.2%) seroprevalence of brucellosis using RBPT reported by Ferede *et al.* (2011) and 4.2% using CFT reported by Ashagrie *et al.* (2011) in Africa. This could be due to geographical difference and management practices as raising of multiple species is practiced in African territories which is a predisposing factor for brucellosis (Ferede *et al.*, 2011).

In the present study seroprevalence of brucellosis varied with area. Similar type of results were observed by

Teshale *et al.* (2006) who declared that seroprevalence rate of brucellosis was significantly higher in Afar region (16%) than Somali region (1.6%) of Eastern Ethopia. Herd practicing may be the reason for this variation in seroprevalence among different areas because in Tehsil Layyah, raising of multiple species is more common, which is an important risk factor for spreading of brucellosis (Kaoud *et al.,* 2010). The findings of this study suggest that seropositivity against *Brucella* was not correlated with sex and these results are comparable to Muma *et al.* (2006). It could be due to sample size herd might not be enough to correlate the disease with sex. Though not significant but the results of present study suggest increasing prevalence rate of disease with increasing age. Kazi *et al.* (2005) also found similar trend in seroprevalence with varying age. High frequency of brucellosis among older animals may be related to maturity with the increasing age (Kazi *et al.,* 2005). As, during early age, pathogen might have proliferated to remain either as latent infection or it may show clinical signs of the disease. Regarding the association between seropositivity against *Brucella* with abortion history, Brucella associated prevalence of abortion came out to be lower in comparison with the result reported by Samadi *et al.* (2010), who reported prevalence rate of 27.1% among animals with history of abortion in Jordanian sheep and goat. While the finding of this study is higher when compared to 13.0% *Brucella* associated prevalence of abortion in Northern Jordan reported by Al-Talafhah *et al.* (2003). Differences in the serological test sensitivity, infection stage, duration and design of study, and variations within infected flocks may be the possible explanation for these variations among different studies (Al-Talafhah *et al*., 2003).

Herd based seroprevalence of the disease in the district was 42.5% and this rate is lower than the one recorded by Al-Majali (2005), who noted a herd prevalence of 53.6% while it was 2.9% in small ruminants in Syria (Darwish and Benkirane, 2001). These variations may be due to different types of grazing and managemental practices as grazing at common pastures and contact with other flocks predisposes the animal to brucellosis (Kadohira *et al.,* 1997). In the current study, no sheep serum sample was found to be positive by indirect and competitive ELISA. Mustafa *et al*. (2011) also could not find any sero-positive sheep by SAT while studying the prevalence of brucellosis in small ruminants in District Lahore. The possible reason for these false positive results by mRB test may be cross reaction of RB antigens with the antibodies other than *Brucella* like *Y. enterocolitica* O:9, *Francisella tularensis*, *Escherichia coli* O:157, *Moraxella phenylpyruvica* and some species of *Salmonella*. Particularly *Y. enterocolitica* O:9 becomes a hurdle in the diagnosis of brucellosis because the immunodominant O-chain of S-LPS of Brucella spp. and *Y. enterocolitica*, serotype O:9 are identical (Chenais *et al*., 2012).

The findings of present study reveal that though appreciable rate of seroprevalence was recorded in the district by one of our serological tests, but none of the test animal proved to be seropositive by using more reliable test. In fact, study on large sample size is required to declare the disease free status of the area.

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