(RESEARCH ARTICLE)

Zoonotic brucellosis: Seroprevalence and different serological tests comparison in ovine and caprine population in district Quetta, Balochistan

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Abstract

The present study was design to determine the seroprevalence of zoonotic brucellosis and different serological tests comparison in ovine and caprine population in district Quetta, Balochistan. A total of 500 blood samples, comprising of 250 each from sheep and goat were randomly collected from out skirts of District Quetta, Balochistan. Out of the 250 blood samples 125 were collected from each males and females. The serum samples were tested for the presence of anti-Brucella antibodies by Rose Bengal Plate Test (RBPT), Serum Plate Agglutination Test (SPAT) and Serum Tube Agglutination Test (STAT). The overall prevalence of brucellosis in sheep was recorded as 16.4%, 13.2% and 10.8% by RBPT, SPAT and STAT, respectively and in goat was found to be 11.6%, 8.8% and 6.8% by RBPT, SPAT and STAT, respectively. The sex-wise prevalence of brucellosis in Ram (male sheep) was recorded as 12.8%, 8.8% and 7.2% by RBPT, SPAT and STAT, respectively; while in Ewe (female sheep) it was 20.0%, 17.6% and 14.4% by RBPT, SPAT and STAT respectively. In Buck (male goat), the seroprevalence of brucellosis was recorded 8.0%, 5.6% and 3.2%, whereas in Dew (female goat) it was 15.2%, 12.0% and 10.4% by RBPT, SPAT and STAT, respectively. The prevalence of brucellosis was relatively higher in ovine (sheep) as compared to caprine (goat), irrespective of techniques used. It was also concluded that brucellosis was higher in females than in males in both sheep and goat. Among all the serological tests applied in the present study, RBPT was found to be more sensitive and showed higher prevalence of brucellosis in sheep as well as in goat population.

Keywords: Ovine; Caprine; Brucella; Seroprevalence; Antibody Titre; Rose Bengal Plate Test; Serum Tube Agglutination Test; Serum Tube Agglutination Test.

1. Introduction

Brucellosis is an important world's major zoonotic bacterial disease of wild and domestic animals (cattle buffalo sheep and Goat) and humans caused by *Brucella abortus*, categorized as re-emerging infectious agent associated with significant morbidity that can lead to increased rates of spontaneous abortions in livestock and also in humans (Cutler et al., 2005: Coelho et al., 2007). Brucellosis is also known as Bang's disease, contagious abortion, infectious abortion, undulant fever, Malta fever and Mediterranean fever. The Brucella species are non-motile, non-sporing, aerobic (but may require 5-10% CO_2 for growth except Brucella suis and Brucella canis), small Gram-negative rods or coccobacilli. The incubation period is usually 1-6 weeks (Blood et al. 1983 and Brooks et al., 1998). The disease is widely considered to be an occupational disease that mainly affects slaughter-house workers, butchers, livestock producers, shepherds, farmers, veterinarians, and laboratory technicians, consumers of unpasteurized milk and other dairy products made from unpasteurized milk is also a major barrier for the trade. (WHO, 1996: OIE, 2012). The organisms become localized in the reticulo- endothelial tissues, namely, the lymph nodes, liver, spleen, kidneys and bone marrow. Within these tissues, the organisms multiply within the macrophages. Whereas in the female the organism localizes in the udder,

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uterus, and lymph nodes adjacent to the uterus leads abortion during the last trimester of pregnancy, retention of fetal membranes (Din et al., 2013).

The disease burden is more profound in the developing countries due to lack of effective public health measures, domestic animal health programs and appropriate diagnostic facilities (Sharifi et al., 2014). The economic losses caused by ovine and caprine brucellosis are mainly attributed to abortions, reduced fertility, increased neonatal losses and to a lesser extent, to orchitis and epididymitis, and has an adverse effect on total animal protein supplies, and severe hazard to human health (Hirsh and Zee, 1999).

Despite its eradication in some countries, brucellosis is still present in the Middle East, Africa, Central Asia and Latin America (Refai, 2002; Coelho et al., 2007). In Pakistan, brucellosis is still remaining one of the major disease problems that affect animal industry as well as human health (Din et al., 2013).

The majority 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 situation about the prevalence of brucellosis in small ruminants in the District is unknown. Therefore, this study was designed to determine the current status of brucellosis in sheep and goat populations, using Rose Bengal Plate Test (RBPT), Serum Plate Agglutination Test (SPAT) and Serum Tube Agglutination Test (STAT) and also to know the species wise and sex wise prevalence of brucellosis in Quetta.

2. Material and methods

2.1. Blood samples collection and Tests detail

A total of 500 blood samples were collected from sheep and goats in the out skirts of Quetta District. Out of total samples 250 were collected from each species including both sexes (Male=125 and Female=125) respectively to observe the seroprevalence of antibodies against Brucella. After aseptically collection of the blood samples from each animal, serum was separated from each sample and transferred to other sterile prelabelled microfuge tubes (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, SPAT and STAT.

2.2. Rose Bengal Plate Test (RBPT)

The test was performed on the method described by Alton et al. (1988) using the antigen, obtained from Veterinary Research Institute, Lahore, Pakistan. A drop of each serum sample and Rose Bengal antigen was added and mixed gently on a clear glass slide. The reaction was observed after few minutes. Results were recorded as: complete agglutination indicated positive, partial agglutination as doubtful and lack of agglutination indicated as negative result.

2.3. Serum Plate Agglutination Test (SPAT)

The SPAT was performed as per the method described by Alton et al. (1988) using the antigen (11% Brucella suspension) stained with crystal violet and brilliant green, obtained from Veterinary Research Institute, Lahore, Pakistan, was further diluted in 0.5% phenol saline (PS). Firstly 160, 80, 40, 20, and 10 μl of the serum sample were placed in a row on 4-cm squares marked on a glass plate. Then, 30 μl of antigen was dropped onto each square and mixed with a spreader in circles, starting with 10 μl of serum and spreading it over an area 2 cm in diameter. The same procedure was used for the other serum dilutions, except that the diameter of the spread was increased up to 3 cm for the 80 μl and 160 μl serum samples. The plate was rotated to ensure proper mixing and allowed to stand for 8 minutes in a testing box, with one gentle rotation 4 minutes after mixing. The testing box having a light source, thrown oblique light on to the serum-antigen mixture plate, painted black, covered with a cover slip to prevent too rapid evaporation. After 8 minutes, the plate was tilted to allow the mixture to flow aside for the reading. The dilutions correspond from 1:10, 1:20, 1:40, 1:80 and 1:60 were classified as positive and abow was classified as suspicious.

2.4. Serum Tube Agglutination Test (STAT)

The STAT was performed as per the method described by Alton et al. (1988) using the plain antigen, procured Veterinary Research Institute, Lahore, Pakistan. In order to perform the test, 0.8 ml of 0.5% phenol saline was taken in the first agglutination tube whereas 0.5 ml of the same was taken in remaining four agglutination tubes placed in a rack. Then after 0.2 ml of serum sample was added in the first tube and mixed well by shaking. The 0.5 ml of diluted serum was transferred from first to the second tube and the process was repeated up to the fifth tube. The 0.5 ml of diluted serum was discarded from the last tube and 0.5 ml of plain antigen was added to each tube to get final dilution of 1:10, 1:20, 1:40, 1:80, and 1:160 in first, second, three, four, and fifth tube, respectively. A control tube was set up to simulate

50% agglutination by mixing 0.5 ml antigen and 1.5 ml of 0.5% phenol saline in an agglutination tube. All six tubes incubated at 37 °C for 20 h before observation. Sera samples showing agglutination at 1:10 or above was considered as to be positive.

3. Results

Generally seroprevalence of brucellosis in sheep was recorded as 16.4%, 13.2% and 10.8%, while in goats was founded to 11.6%, 8.8% and 6.8% by RBPT, SPAT and STAT respectively. Seroprevalence of brucellosis was recorded higher in sheep as compared to goats populations, irrespective of the techniques (Table- 1). Among these serological tests, the RBPT showed higher (16.4% and 11.6%) prevalence of brucellosis in both sheep and goats as compared to SPAT and STAT. The STAT showed lower (10.8% and 6.8%) incidence of brucellosis in both, sheep and goats populations.

Out of 250 sheep the seroprevalence of brucellosis in male sheep was recorded as 12.8%, 8.8% and 7.2% by RBPT, SPAT and STAT respectively, while in female sheep it was 20.0%, 17.6% and 14.4% by RBPT, SPAT and STAT respectively. It is noticeable from the data that a higher prevalence of brucellosis was recorded in female sheep as compared to male sheep (Table 2). Similarly out of 250 goats samples, the seroprevalence of brucellosis in males was recorded as 8.0%, 5.6% and 3.2%, while in females it was recorded as 15.2%, 12.0% and 10.4% by RBPT, SPAT and STAT respectively. Generally it was observed that irrespective of any techniques used in the present study, a higher prevalence of brucellosis was recorded in female as compared to male.

3.1. Antibody titre

During present study serum samples were also examined for antibody titre by using Serum Plate Agglutination Test (SPAT). Out of 250 sheep serum samples, 29 (11.6%) were reactive and showed antibody titre at dilutions of 1:20, 1:40, 1:80 and 1:160. Of these 29 positive reactors, 12 samples showed antibody titre at dilution of 1:20, 8 samples at dilution of 1:40, 6 samples at dilution of 1:80 while 3 samples at dilution of 1:160 (Table 4). Similarly in goats, out of 250 samples, only 19 (7.6%) sera were reacted and showed antibody titre at dilutions of 1:20, 1:40 and 1:80. Among 19 positive reactors, 9 showed antibody titre at dilution 1:20, 6 showed antibody titre at dilution 1:40 while remaining 4 sera showed antibody titre at dilution of 1:80 (Table 4).

3.2. The antibody titre examined by STAT

From 250 sheep serum samples, 25 (10.0%) interacted with antigen and showed antibody titre at dilutions of 1:20, 1:40, 1:80 and 1:160. Out of 25 positive samples, 11 showed antibody titre at dilution of 1:20, 8 showed antibody titre at dilution of 1:40, while 5 samples at dilution of 1:80, however, 1 sample showed antibody titre at dilution of 1:160 (Table 5). In the same way, out of 250 goats serum samples 15 (6.0%) were reactive and showed antibody titre at dilutions of 1:20, 1:40 and 1:80. However, from 15 positive reactors, 8 showed antibody titre at dilution of 1:20, 6 showed antibody titre at dilution of 1:40 while 1 samples at dilution of 1:80 (Table 5).

3.3. The comparative sensitivity of tests

Of 500 serum samples, 70 (14.0%) were found positive by RBPT, 55 (11.0%) by SPAT and 44 (8.8%) by STAT, respectively as shown in Table-6. The RBPT was found to be more sensitive than other two techniques and showed a higher prevalence of brucellosis in sheep as well as in goat populations. The Serum Tube Agglutination Test (STAT) was found less sensitive than Serum Plate Agglutination Test (SPAT) but its results were found to be more reliable because it consists of a proper dilution method and showed qualitative as well as quantitative results about the antibody titre against brucellosis.

Table 1 The seroprevalence of brucellosis in Sheep and Goat populations examined by various serological techniques

Techniques used	Sheep			Goat		
	Total No. of serum samples examined	No. of positive samples	%age of positive samples	Total No. of serum samples examined	No. of positive samples	%age of positive samples
RBPT	250	41	16.4	250	29	11.6
SPAT	250	33	13.2	250	22	8.8
STAT	250	27	10.8	250	17	6.8

Table 2 The seroprevalence of brucellosis in male and female of Sheep investigated

Techniques used	Male (Ram)			Female (Ewe)		
	Total No. of serum samples examined	No. of positive samples	% age of positive samples	Total No. of serum samples examined	No. of positive samples	%age of positive samples
RBPT	125	16	12.8	125	25	20.0
SPAT	125	11	8.8	125	22	17.6
STAT	125	09	7.2	125	18	14.4

Table 3 The seroprevalence of brucellosis in male and female of Goat populations

Techniques used	Male (Buck)			Female (Dew)		
	Total No. of serum samples examined	No. of positive samples	% age of positive samples	Total No. of serum samples examined	No. of positive samples	%age of positive samples
RBPT	125	10	8.0	125	19	15.2
SPAT	125	07	5.6	125	15	12.0
STAT	125	04	3.2	125	13	10.4

Table 4 The antibody titre against brucellosis in positive reactors of Sheep and Goat populations examined by SPAT.

Species examined	Sex	Total No. of serum samples examined	Total No. of positive samples	SPAT Agglutination Titres			
				1:20	1:40	1:80	1:160
SHEEP	Male	125	10	5	3	2	0
	Female	125	19	7	5	4	3
GOAT	Male	125	06	3	2	1	0
	Female	125	13	6	4	3	0

Table 5 The antibody titre against brucellosis in positive reactors of Sheep and Goat populations examined by STAT

Species examined	Sex	Total No. of serum samples examined	Total No.of positive samples	STAT Agglutination Titres			
				1:20	1:40	1:80	1:160
SHEEP	Male	125	09	5	3	1	0
	Female	125	16	6	5	4	1
GOAT	Male	125	05	3	2	0	0
	Female	125	10	5	4	1	0

Table 6 The comparative sensitivity of different serological techniques applied in present the study.

Techniques used	Total No. of serum samples examined	No. of positive samples	% age of positive samples
RBPT	500	70	14.0
SPAT	500	55	11.0
STAT	500	44	8.8

4. Discussion

The prevalence of brucellosis is increasing in Pakistan especially in large sized dairy herds and different serological tests have been used in various studies to find out the incidence of the disease in the country (Abubakar et al., 2012). In the present study, the overall seroprevalence of brucellosis in sheep was recorded to be 16.4% by RBPT, 13.2% by SPAT and 10.8% by STAT respectively. While in goat the prevalence was recorded as 11.6% by RBPT, 8.8% by SPAT and 6.8% by STAT, respectively. Generally, all serological tests showed similar results, however, the RBPT recorded relatively higher prevalence of brucellosis in both sheep and goat as compared to other two serological tests and was found in sequence with the results of Omer et al. (2000) who screened- out the samples from goats and other domestic animals for Brucella infection by Rose Bengal Test at the rate of 14.3% by RBPT in goats. Hunduma and Regassa (2009) studied the prevalence 12.2% in female and 9.8% in male goats using the Rose Bengal Plate Test. The results of the present study are also in line to that of Junaidu et al. (2010) who investigated the seroprevalence of brucellosis by using the Rose Bengal test as 7·24%. The mean value of prevalence as obtained in the present study was in accordance to the range reported by Riaz (2006) as 9.88% by SPAT and 5.88% by STAT. Similar findings regarding the seroprevalence were reported by Wali (2005) who recorded seroprevalence in 2.0% males by SPAT and 13.38% by STAT in female Goats. A study from Pakistan made by Riaz (2006) regarding the seroprevalence as 5.71% males and 12.73% females by SPAT, while by STAT it was recorded as 1.43% and 8.79% in males and females, respectively. Junaidu et al. (2010) also recorded a higher prevalence of brucellosis in female as compared to male goats by using Rose Bengal Plate Test (RBPT), the Serum Agglutination Test (SAT) and the Competitive ELISA (complisa) for brucellosis as in goats, the prevalence of *Brucella abortus* was found to be 6.66 and 5.33% by SPAT and STAT, respectively.

Similarly the seroprevalence in male sheep was recorded as 12.8%, 8.8% and 7.2% by RBPT, SPAT and STAT respectively, while in female sheep it was showed as 20.0%, 17.6% and 14.4% by RBPT, SPAT and STAT respectively. However Hussain et al. 2014, Iqbal et al. 2013 and Negash et al. 2012 recorded the seroprevalence of ovine brucellosis as 10.0%, 8.07% and 7.0% in Kohat, Ethiopia and Southern Punjab (Pakistan), respectively. Lone et al. (2013), reported the prevalence of ovine brucellosis as 6.50% in Kashmir. This slightly higher prevalence in the current study on brucellosis could be attributable to geographical variation and altered systems of vaccination and management. However, the seroprevalence of sheep brucellosis revealed from the current study to be much lower than reported by Hamidullah et al. (2009), who found 34.8% sheep positive to Brucella in Kohat. Extensive animal farming has been documented as a potential risk factor for ovine brucellosis (Al-Majali, 2005; Yesuf et al., 2010).

The similar seroprevalence of brucellosis in ewes was recorded by (Hussain et al. 2014) as 12.00%, 10.00% and 10.00% through SAT, RBPT and MRT, respectively. The prevalence of brucellosis was relatively higher in ewes with RBPT and MRT as compared to Rams, SAT and RBPT recorded the seroprevalence as 8.0% and 10.0%, respectively by (Hussain et al. 2014). The Kotadiya A.J. (2012) reported higher seroprevalence by RBPT (11.38%) and least by STAT (9.44%) in sheep of Gujarat. The similar results have also been reported by (Yesuf et al., 2010; Negash et al., 2012; Rahman et al., 2013). The presence of erythritol in allantoic fluid favours the growth and propagation of Brucella organisms thereby enhancing the susceptibility of female sheep to brucellosis (Yesuf et al., 2010; Rahman et al., 2011).

In the current study, seroprevalence of brucellosis in sheep was recorded as 13.2% and 10.8% while in goats was founded to 8.8% and 6.8% by SPAT and STAT respectively. Seroprevalence of brucellosis was recorded higher in sheep as compared to goats populations. Among these serological tests, the SPAT showed higher (13.2% and 8.8%) prevalence of brucellosis in both sheep and goats as compared to STAT. The STAT showed lower (10.8% and 6.8%) incidence of brucellosis in both, sheep and goats populations respectively. The study results were comparable to Negash et al. (2012) who reported the prevalence of brucellosis in ovine to be 8.7% in Ethiopia. Slightly 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).

5. Recommendations

After the facts and figures of this study, the author recommended the following preventive measures to reduce the prevalence of brucellosis in sheep and goats in the area in particular and in the country in general. A frequent serological examination must be carried-out to know the prevalence of brucellosis in all animals and the suspected/infected should be separated/slaughtered immediately. While dealing with brucellosis, veterinarian should take care and adopt all protective measures so as to make sure that all contaminated or infected materials such placenta, aborted fetus etc. should be destroyed/buried properly to reduce chances of spreading the infection in animals as well as in humans. Milk must be pasteurized before consumption as it is a potential source of infection from animals to human beings. Proper awareness regarding brucellosis in animals and infection related risk factors to human health, zoonotic importance, disease control status/prevention programs and its economical losses in terms of reduced livestock production should be provided through mass media to public to help in its eradication from the area.

6. Conclusion

On the basis of the present study, it is concluded that the brucellosis is prevailing in sheep and goat of the area as determined by different techniques. The bacterial specie *Brucella abortus* was identified as the only specie causing brucellosis in sheep and goat. Sex-wise, it is recorded that brucellosis was relatively higher in females than in males in both sheep and goat. Further, seroprevalence of brucellosis was significantly more frequent in sheep as compared to goats. Among the three serological tests, the RBPT showed highest seropositivity for brucellosis in both sheep and goats followed by SPAT and STAT. The Serum Tube Agglutination Test (STAT) was found less sensitive than Serum Plate Agglutination Test (SPAT) but its results were found to be more reliable because it consists of a proper dilution method and showed qualitative as well as quantitative results about the antibody titre against brucellosis. The serum antibody titre of *Brucella abortus* was determined which interacted with antigen at dilutions of 1:20, 1:40, 1:80 and 1:160. However, beyond these dilutions, no interaction between antigen and serum antibodies was observed.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest associated with this research work.

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