Research Article

Caprine and Ovine Serological Evidence of Brucellosis in Five Districts of Punjab, Pakistan

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Abstract | Brucellosis is a worldwide zoonotic disease that affects various animal species. Amongst different *Brucella* species, *Brucella melitensis* has the greater zoonotic aspect, hence, it is considered an occupational hazard for small ruminant handlers as well as Veterinarians. The present study reports the seroprevalence of brucellosis in different sheep and goat breeds in five districts of Punjab, Pakistan. A total of 1239 serum samples were collected from male (n=73) and female (n=1166) different sheep (n = 865; Pak-Karakal, Thalli, Lohi, Kajli and non-descript type) and goat (n = 374; Teddy, Beetal and non-descript type) breeds of variable age groups (0 months to 4years). All the serum samples were analyzed using Rose Bengal Plate Test (RBPT) and seropositive samples were further submitted to Serum Agglutination Test (SAT). The results revealed that the prevalence of brucellosis was higher (P<0.05) in goats than sheep, irrespective of test used. The serological prevalence of brucellosis differs significantly (P<0.05) among sheep and goat breeds, and Thalli sheep and Teddy goats had higher (P<0.05) seropositivity compared to the other sheep or goat breeds. The prevalence was relatively higher (P<0.05) in male animals than female animals, regardless of species. However, sheep or goat age groups did not influence (P>0.05) the brucellosis seroprevalence. Among seropositive animals, significantly greater (P<0.05) number of animals had previous history of abortion when tested either by RBPT or SAT. In conclusion, the current data provide baseline information about prevalence of brucellosis in different sheep and goat breeds in five districts of Punjab Pakistan.

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Introduction

Brucellosis is a highly important zoonotic disease that spread through gram negative, non-encapsulated, small (0.5 to 0.7 by 0.6 to 1.5 µm), nonmotile, facultative and intracellular bacteria of genus *Brucella* (Saxena and Raj, 2018). There are different species of *Brucella* that affect variety of the animals such as *B. melitensis* (mainly infecting ovine and caprine), *B. abortus* (mainly infecting bovines), *B. Ovis* (mainly infecting ovine) and *B. suis* (mainly infecting swine) and these *Brucella* species have gain more recognition due to their zoonotic characteristics (Godfroid *et al.*, 2011; Blasco and Molina-Flores, 2011). Though, the transmission from human to human is rare but it is possible to spread either from infected mother to neonates or sexual intercourse (Saxena and Raj, 2018).

Threatened abortions, reduced fecundity rate and decrease production are the major associated problems to brucellosis. Although, the small ruminants are highly populous and prolific in Punjab province as compared to other species; however, the optimum production have not been achieved due to poor nutrition, improper housing, lacks of breeding plans and high infectious diseases incidence (FAO, 2011). Incidence of infectious disease in sheep and goat results in high mortality, poor production and low reproduction performance (Anaeto *et al*., 2009). In this context, the reproductive diseases severely affect the production capacity of farm animals. Among infectious diseases, Brucellosis is one of the major reproductive disease which affects the fertility and prolificacy in small ruminants due to excess abortion and endometritis (Blasco and Flores, 2011). Due to zoonotic aspect of *B. melietensis* species, there are always high concerns regarding the health of farmers and concerned staff dealing with small ruminants. (Hunter and Kreeger, 1998; Samartino, 2002; Roth *et al*., 2003; Santos *et al*., 2013). Therefore, serological evidence is necessary to adopt control strategies for brucellosis in livestock and humans due to its zoonotic aspect (Garin-Bastuji *et al*., 1998).

Brucella transmission is also possible through direct contact with contaminated placenta, aborted embryos, retained fetal membranes and other reproductive secretions (Franc *et al*., 2018). A lot of efforts and strategies have been adopted to control the risk of brucellosis in small ruminants; however, the outputs are not encouraging because of sheep and goat rearing systems, uncontrolled transportation of animals especially during the festivals, use of infected males for natural breeding rather than AI, are key risk factors for reemergence of disease in this region.

There are numerous available serological tests for brucellosis detection but screening of large herds for brucellosis is expensive and time consuming. In this scenario, selection of authenticated tests like Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT), is important for diagnosis because RBPT and SAT tests have been comprehensively validated and widely practiced in different species (Blasco *et al*., 1994; Lucero and Bolpe, 1998; Barroso *et al*., 2002; Munoz *et al*., 2005; Garin-Bastuji *et al*., 2006). The literature regarding prevalence of brucellosis is abundantly available in different farm animals from different regions of world. However, it is the dire need of time to produce much safer food for human beings through screening of brucellosis and to generate basal information about prevalence of brucellosis for control the infection in livestock species. Therefore, the present study was conducted with the aim to find the prevalence of brucellosis in different sheep and goat breeds out of five selected districts of Punjab, Pakistan.

Materials and Methods

Study area

Blood samples were collected from flocks of small ruminants maintained at three Government farms; Livestock Experimental Stations Aladad Jahanian, District Khanewal (30.2864° N, 71.9320° E), Livestock Experimental Station Rakh Khaire Wala, District Layyah (30.9693a, ion Rakh Khaire Wala, of small ruminants maintaiKhizerabad, District Sargodha (32.0740° N, 72.6861° E). Additionally, two private livestock farms were selected for sampling; Malik Goat Farm District Faisalabad (31.4504sampling;haire Wala, Mumtaz Watto Sheep Farm District Okara (30.8138° N, 73.4534° E). The climatic conditions (ambient temperature, relative humidity and precipitation) of study area have been shown in the Table 1.

Table 1: *Climate of experimental sites during sample collection period Aug-Oct 2010.*

Districts	Avg. temp	RH (%)	Avg. rainfall (mm)
Khanewal	30.66	41.66	10.76
Layyah	31	39	15.13
Sargodha	30.33	46	16.44
Faisalabad	30.33	47.33	16.69
Okara	30	49	15.19

Description of animals and data collection

A total 1239 blood samples were collected from sheep (Pak Karakul, Lohi, Kajli and Thalli breed and non-descript type of sheep) and goat (Teddy and Beetal breed and non-descript type of goats). The selected sheep and goats were from both male (n=73) and females (n=1166) genders. Later the animals were further categorized into different age groups i.e. zero to 6 months, 6 months to 2 years, 2 to 4 years and above 4 years. A total of 307 blood samples were obtained from Livestock Experimental Station Aladad Jahania which includes 190 Lohi sheep and 117 Beetal goats. A sum of 175 blood samples of Kajli sheep

were obtained from Livestock Experimental Station Khizarabad, Sargodha and 530 blood samples were obtained from Livestock Experimental Station Rakh Khairewala which includes 155 Thalli sheep, 245 Pak Karakal sheep and 130 Teddy goats. In addition, 100 and 127 samples were collected from non-descript type of sheep and goats kept by local livestock farmers in the periphery of Faisalabad and Okara districts. Moreover, required clinical, epidemiological and reproductive information were also recorded. During sampling a questionnaire-based information on age, sex, pregnancy status, disease history, reproductive problems such as abnormal uterine discharge, abortion and reproductive diseases were also recorded. All procedures performed involving live animals in this study were in accordance with the ethical standards laid down by Faculty of Veterinary Science and animal welfare policies.

Sample collection

About 6 ml blood from the jugular vein was gently collected in 10 ml disposable sterilized plastic syringe and the blood was allowed to clot in a slanting position at least for 1 hr. Later, it was kept in refrigeration for overnight. The next day serum was collected in disposable screw caped plastic bottles and transported to Theriogenology lab, University of Agriculture Faisalabad. The samples were stored at -20°C before further processing. Hyper immune sera were raised to run control positive and control negative tests along with the serum samples to be tested. For this purpose, two rabbits were injected (ear vein) with known *Brucella abortus* concentrated antigen procured from Veterinary Research Institute, Lahore.

Laboratory tests

Rose bengal plate test (RBPT): Serum of 30 µl by using micropipette was mixed with an equal volume of antigen on a glass slide produce a zone approximately equal to 2 cm in diameter. The mixture was mixed gently with disposable sterile stirrer for four minutes at ambient temperature and then observed for agglutination. Any visible reaction was graded positive and otherwise negative (OIE, 2003).

Serum agglutination test (SAT): Serum samples found seropositive with RBPT, were further confirmed by SAT. Serum agglutination test (SAT) was run as per standard procedures described by (Stemshorm *et al.*, 1985). Briefly, 0.8 ml of phosphate buffer saline (PBS) containing 0.5% phenol was added in clear glass tubes of approximately 2 ml volume. A 0.2 ml of test serum was added to first tube, mixed and then 0.5 ml was transferred to the second tube. After mixing well, 0.5ml was transferred to the third and continued these steps mixing and transferring up to the last fifth tube. An equal volume (0.5 ml) of standardized for *B. abortus* antigen with phenol saline dilution (1:20) was added and the tubes were incubated overnight at 37°C. The results of agglutination in SAT test tubes were determined by reading the degree of sedimentation in the tubes. A titer of 1:40 or more was considered as positive, titer of 1:20 was considered a doubtful and the titer of 1:10 was treated as negative.

Statistical analysis

The statistical analysis was performed using SPSS software version 15.0 (SPSS Inc., Chicago, Ill, USA). The sex, species and abortion rate were analyzed by chi-square test and data for breeds and age groups were compared by binary logistic regression analysis. Group differences were considered significant at $p<0.05$.

Results and Discussion

The results obtained through RBPT and SAT revealed that the goats were more ($P < 0.05$) seropositive (19.5% and 9.9%) than sheep (7.1% and 3.2%) (Table 2). Seropositivity was also influenced ($P < 0.05$) by different sheep and goat breeds as Thalli sheep among sheep breeds and Teddy among goat breeds, showed higher rate ($P < 0.05$) of *Brucella* seropositivity (Table 3). There was also significant difference ($P < 0.05$) in seroprevalence between male and female animals (Table 4). In contrast, the seroprevalence of Brucellosis was not differed ($P > 0.05$) among all experimental age groups (Table 5). Animals with previous history of abortion were more seropositive than animals with no earlier record for abortion (Table 6).

Table 2: *Overall seroprevalence of brucellosis in sheep and goats of Punjab Pakistan.*

Species	RBPT Positive (%)	SAT Positive (%)
Sheep	61 (7.1)	28 (3.2)
Goat	73 (19.5)	37 (9.9)
	0.000	0.000

P > 0.005 indicate the significance.

It has been observed while analyzing results of this study that there was higher incidence in goats than

sheep. More specifically, Thalli sheep and Teddy goats were found the most infected breeds, whereas male animals were found more prone to infection and all age groups were equally at risk of brucellosis.

Table 3: *Seroprevalence of brucellosis in different sheep breeds of Punjab Pakistan.*

Sheep breeds	RBPT positive (%)	SAT positive (%)
Pak-Karakal	13(5.31)	7(2.85)
Thalli	44(28.39)	18(11.61)
Lohi	3(1.58)	2(1.05)
Kajli	0(0)	0(0)
Non-descript	1(1)	1(1)
p-value	0.000	0.000

Table 4: *Seroprevalence of brucellosis in different goat breeds of Punjab Pakistan.*

Goat breeds	RBPT positive (%)	SAT positive (%)
Beetal	18(15.38)	11(9.40)
Teddy	51(39.23)	24(18.46)
Non-descript	2(1.57)	2(1.57)
p-value	0.000	0.000

Table 5: *Seroprevalence of brucellosis in male and female sheep and goats.*

Sex	RBPT positive (%)	SAT positive (%)
Male	17(23.28)	9(12.32)
Female	115(9.86)	56(4.80)
p-value	0.001	0.011

Table 6: *Seroprevalence of brucellosis in different age groups of sheep and goats.*

Age	RBPT positive (%)	SAT positive (%)
0-6 months	4(4.54)	1(1.13)
6 month-2 year	13(9.55)	5(3.67)
2 year-4year	29(9.00)	15(4.65)
>4 year	86(12.42)	44(6.35)
p-value	0.073	0.128

Table 7: *Seropositivity for Brucella in previously aborted sheep and goats.*

	Seropositive	
	RBPT No. (%)	SAT No. (%)
Animals with abortion history	26(36.61)	17(23.94)
Animals without abortion history	106(9.07)	46 (3.93)
p-value	0.000	0.000

In the present study, the goat breeds were more *Brucella* seropositive than sheep breeds. Previous literature also endorsed that goats were more susceptible to *B. Melitensis* infection than sheep (Quinn *et al.*, 2004; Rahman *et al.*, 2011). In contrast, some reports indicated higher incidences of Brucellosis in sheep (Singh *et al.*, 1998). Earlier reports from Pakistan also showed the variability in incidence of Brucellosis in sheep (Shafee *et al.*, 2011; Hussain *et al.*, 2014) and goats (Din *et al.*, 2013). In this study the reason of high seropositivity in goats might be because of Teddy bucks with more *Brucella* infection, whereas variability about brucella prevalence in different studies might be associated with geographic location, type of diagnostic test, husbandry and environmental factors (Amin *et al.*, 2005).

Thalli amongst sheep and Teddy amongst goat breeds had higher seropositivity for brucellosis. This might be attributed to a factor that animals of these breeds were smaller in size and abundantly present throughout the province of Punjab. High incidence in these breeds might be related to geographical proximity and intermixing to livestock species. Although, the sera samples were obtained from organized farms; however, interaction of different species during grazing could be one of the dominant factors for high brucellosis prevalence. In addition, there is paucity of information regarding the etiology, identification and disease dynamics within breeds and species in different agro-ecological zones of the country. Teddy breed is faster in growth than other breeds, up till now there is no data available which shows the correlation between the growth rate and the disease susceptibility, so a comprehensive study about this possible correlation is recommended.

In current study, high incidence of brucellosis was observed in male gender of both species (23.28% and 12.32%) compared to that of female (9.86% and 4.80%) through RBPT and SAT respectively. The higher incidence in male gender was also supported in a previous study by Saeed *et al.* (2019) that seroprevalence was higher in male animals (7.4%) than in female animals (2.5%). However, Ali *et al.* (2015) reported contrary findings in same species in the Potohar Plateau, (Rawat, Kherimurat and Islamabad) where low seroprevalence was recorded in males (3.03%) as compared to female (10.4%). Another study by Rivera *et al.* (2007) revealed seroprevalence for small ruminants (male 5%, female

9%) in Pakistan. The possible reason for the high seroprevalence in males might be attributed to a factor that farmers could not adopt to cull seropositive animals and allowed to breed. Moreover, higher rate of incidence in this study might be due to practice of natural breeding of seropositive males and exposure of newly introduced male to the infected female. Moreover, submission of larger population of male could reveal better understanding of potential role *Brucella* transmission by breeding males.

Incidence of infection in all age group determines the constant presence of seropositive animals in the herd or latency for an indefinite period prior to clinical manifestation. In contrast, earlier studies showed that older animals were more susceptible to the infection than young one which could be due to low resistance against infection, greater exposure of older animals to infected animals and hormonal dynamic in sexually mature animals (Boukary *et al*., 2013; Akbarian *et al*., 2015). The divergence of present study regarding age factor from previous literature couldn't be ascertained and recommended for further research.

Abortion at an advanced stage of pregnancy might be a major sign of Brucellosis in breeding animals (Harbord *et al*., 2009). In current study, animals which have had history of abortion were diagnosed with higher seropositivity (36.61% and 23.94%) as compared to animals having no history of abortion (9.07% and 3.93%) through RBPT and SAT, respectively. Similar results were also shown in previous research carried out in Uganda (Makita *et al*., 2011), Pakistan (Ali *et al*., 2015), and Kenya for cattle (Muendo *et al*., 2011). The higher incidence in aborted animals might be due to factor of not culling *Brucella*-seropositive animals, thus enabling infected animals to transmit their infection to other healthy animals. In this study, the presented information about abortion/stillbirth has been included in view of targeted breeds; the ratio of abortion could be variable if whole population from a particular area is considered. The current findings provide a representative overview of the association of abortion and seropositivity for *Brucella* infection among sheep and goats in the study area.

Conclusions and Recommendations

In conclusion, the present findings provide baseline information regarding seroprevalence of brucellosis in sheep and goat breeds in five districts of Punjab Pakistan, and these data could be an initiative for the control of brucellosis and to study further the molecular aspect of *Brucella* infections. Future studies are needed to distinguish the high to low incidence in different agro-ecological zones for control strategy and to highlight the possibly linked risk factors of brucellosis incidence.

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Novelty Statement

For the first time, an extensive study on brucellosis was conducted in eight different species of small ruminants and five districts of Punjab. The targeted area of study was selected by keeping in view to cover almost all zones (upper, central & south) of Punjab so that future studies can be possible to distinguish the high to low incidence in different agro-ecological zones for control strategy and to highlight the possibly linked risk factors of brucel-losis incidence

Author's Contribution

MS and MN designed the study, collected samples and performed the experimental work. MS, MF and ZN analyzed the data. MS, MN, MF and ZN wrote the manuscript. AYQ, AR, AK, IK and AuR critically reviewed the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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