Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Sero-Prevalence of Brucellosis in Food Animals in the Punjab, Pakistan

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ARTICLE HISTORY (14-248)

Received: May 17, 2014
Revised: June 13, 2014
Accepted: June 19, 2014

Key words:
Brucellosis
c-ELISA
Food animals
i-ELISA
RBPT
SAT
Sero-prevalence

ABSTRACT

Aim of the present study was to know the prevalence of brucellosis in food animals in relation to various risk factors through different diagnostic tests. For this purpose, 2375 serum samples were collected from December, 2010 to December, 2012. Rose Bengal Plate Test (RBPT) and Serum Agglutination Test (SAT) were applied for initial screening, positive samples were subjected to enzyme linked immunosorbant assays (i-ELISA and c-ELISA) for confirmation. The data thus collected was interpreted and subjected to Binary Logistic Regression Analysis to know the difference among various groups based on species, sex, age, body weight and parity. Through RBPT and SAT, the higher prevalence of caprine brucellosis was recorded as compared to the buffaloes, camel, cattle and sheep. Prevalence of brucellosis was higher in buffaloes through both types of ELISAs as compared to other food animals. No animal was found positive for brucellosis in camel population. The prevalence of brucellosis was not associated with sex of animals. Sero-prevalence was higher in mature animals as compared to the younger ones in all food animals. The other risk factors like body weight and parity also affected the sero-prevalence of brucellosis in all species.

To Cite This Article: Gul ST, A Khan, F Rizvi and I Hussain, 2014. Sero-prevalence of brucellosis in food animals in the Punjab, Pakistan. Pak Vet J, 34(4): 454-458.

INTRODUCTION

Brucellosis, is an important zoonotic disease after rabies, causes significant reproductive losses in sexually mature animals and is a major barrier for the trade (Lopes *et al.,* 2010; Abubakar *et al.,* 2012). Although, brucellosis has been controlled or eradicated in many developed countries; yet it still remains an uncontrolled problem in regions of high endemicity such as the Mediterranean, Middle East, Africa, Central and Latin America, Eastern Europe, Caribbean and parts of Asia (Maurin and Maurin, 2005). 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. As no characteristic constellation of symptoms and signs exists, the diagnosis is usually missed (Gul and Khan, 2007).

From public health view point, brucellosis is one of the world's major zoonotic problems, categorized as class B bioterrorist agent and accounting for the annual occurrence of more than 500,000 cases (Seleem *et al.,* 2010). This disease is considered to be an occupational disease that mainly affects slaughter-house workers, butchers, livestock producers, shepherds, farmers, veterinarians, and laboratory technicians (Behzadi and Mogheiseh, 2011). This disease has been imported from brucellosis-endemic countries into non-endemic areas due to increase in business and leisure travel (Gwida *et al.,* 2010).

The diagnosis of brucellosis is usually performed by a combination of serological and molecular methods. Definitive diagnosis is usually carried out through isolation and identification of the causative organism, but drawback is that it is time-consuming, must be performed by highly skilled personnel, and is hazardous. For these reasons, serological tests like RBPT and serum agglutination test are normally preferred. For confirmation of findings of RBPT and SAT, ELISA based tests are used (Gul and Khan, 2007; Poester *et al.,* 2010).

In Pakistan, the prevalence of brucellosis has been reported to vary from 0 to 32.5% and work has been carried out on the seroprevalence of brucellosis in almost all domestic species like cattle and buffaloes (Asif *et al.,* 2009), sheep and goats (Ghani *et al.,* 1995; Iqbal *et al.,* 2013), camel (Nasrin *et al.,* 1998), horses (Gul *et al.,* 2013) and humans (Mukhtar and Kokab, 2008; Asif *et al.,*

2014). Recently, it was observed that the prevalence of brucellosis in animals is increasing day by day (Abubakar *et al.,* 2012). Mostly, this prevalence is based on RBPT and SAT. No further confirmation has been made. However, serological cross reactions have been demonstrated between *Brucella* species and other bacteria. The present project, therefore was planned to investigate the sero-prevalence of brucellosis in food animals through basic serological reactions and also through latest tests like i-ELISA and c-ELISA.

MATERIALS AND METHODS

Experimental animals: For this study, 2375 food animals including cattle (n=475), buffaloes (n=212), sheep (n=1306), goats (n=282) and camels (n=100) of both sexes were selected randomly from different Government farms in Punjab, Pakistan from December, 2010 to December, 2012. Two well-organized private farms of cattle and buffalo were also included in this study. About 5ml blood without anticoagulant was collected from above mentioned different food animals, serum was separated and stored at –20˚C till analysis for sero-diagnosis.

Sero-diagnosis: The Rose Bengal plate test (RBPT) and serum agglutination test (SAT) were performed following the procedure described by Aldomy *et al*. (2009). The i-Elisa (*Brucella* i-ELISA Antibody Test, Kit # 10-2700-10) and c-ELISA (*Brucella* Ab C-ELISA Test, Kit # 10-2701-10) were performed by following the procedures described by the manufacturers (Brucellosis commercial ELISA kit manual procured from Svanova, Sweden). Various risk factors like species, age, sex, body weight and parity were considered to affect the prevalence of brucellosis.

Statistical analysis: Animals were divided into different groups based upon species (cattle, buffaloes, goats and sheep), sex (female, male), age {cattle (1-7, 8-14 and above 14 years), buffaloes (1-5, 6-10 and above 10 years), goats and sheep (0-12, 13-24, 25-36 and above 36 months)}, body weight (cattle, 100-300, 301-600 and above 600kg; buffaloes, 200-600 and 601-1000kg; goats, 10-30 and 31-60kg; and sheep, 10-40, 41-80kg) and parity (cattle and buffaloes, 0-5 and 6-10; goats and sheep, 0-3 and 4-7). Binary logistic regression analysis was applied through a statistical software MINITAB 16.0 version to know the difference in sero-prevalence among different groups on the basis of all four diagnostic tests applied.

RESULTS

Overall sero-prevalence of brucellosis: Overall sero-prevalence of brucellosis in different food animals was 12.29 and 4.58% through RBPT and SAT, respectively. Initial screening through RBPT showed that highest sero-prevalence was in goats, followed by buffaloes, cattle and sheep. Sero-prevalence of brucellosis through SAT was 8.49, 7.57, 9.57 and 2.14% in buffaloes, cattle, goats and sheep, respectively (Table 1). The statistical analysis indicated that difference in sero-prevalence among various species was statistically significant ($P<0.001$) through RBPT and SAT. Odds ratio indicated that chances of brucellosis sero-prevalence were higher in goats and lower in cattle and sheep as compared to buffaloes through both tests (Table 1).

The i-ELISA based sero-prevalence of brucellosis was 8.01, 16.31, 0, 6.73 and 1.76% in buffaloes, cattle, camels, goats and sheep, respectively and the difference among these species was statistically significant (Table 2). The difference in sero-prevalence on the basis of c-ELISA was also significant among different species. c-ELISA based sero-prevalence was 8.01, 6.94, 6.73 and 1.91% in buffaloes, cattle, goats and sheep, respectively. Odds ratio indicated that chances of brucellosis sero-prevalence were lower in cattle, goats and sheep as compared to buffaloes through both tests (Table 2).

Sex based sero-prevalence of brucellosis: In relation to sex, sero-prevalence of brucellosis in cattle was higher in females as compared to males, while in buffaloes, it was higher in males than females and the differences based upon sex in case of cattle and buffaloes were statistically non-significant through all four tests (Table 3). In goats, prevalence of brucellosis was higher in bucks as compared to does and the difference was statistically significant through all tests except RBPT. In sheep, sero-prevalence of brucellosis was lower in ewes as compared to rams and the difference was statistically significant ($P<0.001$) through all four tests (Table 3).

Age based sero-prevalence of brucellosis: In cattle, **s**ero-prevalence of brucellosis was higher in sexually mature animals as compared to younger animals through all four diagnostic tests. But, the difference among different age groups was statistically non-significant. In buffaloes, sero-prevalence of brucellosis was also higher in sexually mature animals as compared to younger animals through all four diagnostic tests and the difference among three different groups was statistically significant on the basis of all tests (Table 4).

Sero-prevalence of brucellosis in small ruminants was higher in mature animals as compared to younger animals as it was observed in large animals. In goats, the difference in sero-prevalence among these four age groups was statistically significant ($P<0.002$) through RBPT, while it was non-significant through all other tests. In sheep, the difference among different age groups was statistically non-significant through all tests except c-ELISA, where difference was statistically significant ($P<0.04$) (Table 4).

Body weight based sero-prevalence of brucellosis: The difference in sero-prevalence of brucellosis in cattle was statistically significant among three body weight groups depending upon all the four diagnostic tests (Table 5) and highest prevalence was recorded in animals having >600 kg body weight as compared to the other two groups. While, in buffaloes it was non-significant through all the tests. In goats and sheep, statistically the difference among two groups was significant only through RBPT (Table 5) and prevalence was higher in animals having higher body weights.

Table 1: Sero-prevalence of brucellosis in food animals through RBPT and SAT

Species	Total Animals	Rose Bengal Plate Test			Serum Agglutination Test		
		Positive (%)	Coefficient±SE	Odds Ratio	Positive (%)	Coefficient±SE	Odds Ratio
Buffalo	212	27 (12.73)	-1.924±0.206	-	18 (8.49)	-2.377±0.246	-
Cattle	475	49 (10.32)	-0.238±0.255	0.79	36 (7.57)	-0.123±0.301	0.49
Goat	282	99 (35.10)	1.310±0.240	3.71	27 (9.57)	0.132±0.318	0.61
Sheep	1306	117 (8.95)	-0.394±0.227	0.67	28 (2.14)	-1.443±0.311	0.13
		Chi-square Value	125.095			41.917	
		P-value	0.001			0.001	

Values in parenthesis indicate percentage.

Table 2: ELISA based sero-prevalence of brucellosis in food animals

Species	Total Animals	i-ELISA			c-ELISA		
		Positive (%)	Coefficient±SE	Odds Ratio	Positive (%)	Coefficient±SE	Odds Ratio
Buffalo	212	17 (8.01)	-2.439±0.252	-	17 (8.01)	-2.439±0.252	-
Cattle	475	30 (6.31)	-0.257±0.315	0.77	33 (6.94)	-0.155±0.310	0.86
Goat	282	19 (6.73)	-0.187±0.346	0.83	19 (6.73)	-0.187±0.346	0.83
Sheep	1306	23 (1.76)	-1.581±0.328	0.21	25 (1.91)	-1.496±0.323	0.22
		Chi-square Value	33.5135			33.4682	
		P-value	0.001			0.001	

Values in parenthesis indicate percentage.

Parity based sero-prevalence of brucellosis: Sero-prevalence of brucellosis was higher in animals having 6-10 parity as compared to those having 0-5 parity in cattle and buffaloes, the difference was statistically significant ($P<0.004$) through RBPT only in cattle, but it was non-significant through all other tests in animals of the two species (Table 6).

In goats, sero-prevalence of brucellosis in two groups based upon parity was higher in animals having 4-7 parity as compared to those having 0-3 parity the difference between these two groups was statistically significant on the basis of all diagnostic tests, except RBPT (Table 6). Difference in sero-prevalence of ovine brucellosis in two groups based upon parity was statistically non-significant (Table 6). However, prevalence of brucellosis in ewes having more than 4 lactations was more as compared to those having less than 4 number of lactations. Parity based results indicated that animals having more parity were more prone to the brucellosis.

DISCUSSION

Brucellosis is a highly contagious bacterial disease which is not only of zoonotic importance but is also a disease of economic importance. It adversely affects the productive and reproductive potential of animals in terms of reduction or complete cessation of milk production after abortion, loss of young ones and temporary or permanent infertility (Gul and Khan, 2007; Shabbir *et al*., 2013).

Overall sero-prevalence of brucellosis in different food animals was 12.29 and 4.58% through RBPT and SAT, respectively. The highest sero-prevalence of brucellosis was recorded in goats, followed by buffaloes, cattle and sheep on the basis of RBPT and SAT. The odds ratio indicated that the brucellosis occurrence chances were higher in goats as compared to the other species. The published literature also indicated that the prevalence of caprine brucellosis was higher as compared to the other types of brucellosis (Akbarmehr and Ghiyamirad, 2011). It might be due to fact that in husbandry practices, these animals are usually kept overcrowded and reared in open system with different ages and without differentiation of aborted and pregnant ones and even males and females are housed together with high stocking density, all these factors play important role in the spread of the infection. Another reason could be that in traditional farming, farmer does not have knowledge about brucellosis and usually keep *Brucella* infected animals for breeding purpose which serves as source of infection.

Through i-ELISA, the highest sero-prevalence was recorded in buffaloes, followed by goats, cattle and sheep. The i-ELISA based sero-prevalence of brucellosis in cattle was lower than the recently reported prevalence in Pakistan which was 20%, but in buffaloes it was higher which was 0.0-7.74% (Abubakar *et al.,* 2010). Overall prevalence of brucellosis in camel population was recorded 0% which is in accordance to the previous results in which prevalence of brucellosis in camel had been reported to be 0.0-17.20% (Gul and Khan, 2007). A wide variation in brucellosis prevalence among different food animal species could be due to difference in prevalence of the disease in that geographic region, diagnostic tests applied, close contact with infected domestic and wild animals, population intensity or husbandry system being practiced (Sikder *et al.,* 2012; Gul *et al.,* 2013).

In this study, a non-significant difference was observed in sero-prevalence of brucellosis on the basis of sex in bovines and it was in accordance to the results previously reported that the prevalence of brucellosis appeared not to be associated with sex and disease prevalence was as frequent in males as in females (Akbarmehr and Ghiyamirad, 2011; Asmare *et al.,* 2013). In case of caprine and ovine brucellosis, prevalence was higher in males as compared to females as has also been expressed by Rahman *et al.* (2011). However, these results contradict with previous reports, where it was stated that prevalence in females was significantly higher than males (Khan *et al.,* 2009; Omer *et al.,* 2010; Junaidu *et al.,* 2011).

In the present study, sero-prevalence of brucellosis was higher in sexually mature animals as compared to younger animals in buffaloes. The same was true in case of caprine brucellosis on the basis of RBPT and in ovine brucellosis on the basis of c-ELISA. These results are in accordance to the previous reports which stated that the prevalence of brucellosis appears to be associated with

Table 3: Sex based sero-prevalence of brucellosis in food animals (%)

Test	Sex	Species			
		Cattle	Buffaloes	Goats	Sheep
RBPT	Female	10.65	12.37	34.27	8.25
	Male	0	20	41.17	24.1
	P-value	0.068	0.508	0.434	0.001
SAT	Female	7.82	8.41	7.25	1.52
	Male	0	10	26.47	15.51
	P-value	0.121	0.864	0.002	0.001
i-ELISA	Female	6.52	7.92	4.83	1.36
	Male	0	10	20.58	10.34
	P-value	0.158	0.819	0.002	0.001
c-ELISA	Female	7.17	7.92	5.24	1.44
	Male	0	10	17.64	12.06
	P-value	0.138	0.819	0.019	0.001

Table 4: Age based sero-prevalence of brucellosis in food animals (%)

Species	Age Groups	Test			
		RBPT	SAT	i-ELISA	c-ELISA
Cattle	1-7	7.48	7.08	5.51	6.29
	8-14	13.17	7.80	7.31	7.80
	Above 14	18.75	12.5	6.25	6.25
	P-value	0.078	0.751	0.733	0.816
Buffaloes	1-5	6.75	2.70	1.35	1.35
	6-10	12.12	10.10	10.10	10.10
	Above 10	25.64	15.38	15.38	15.38
	P-value	0.023	0.038	0.009	0.009
Goats	0-12	7.69	0	0	0
	13-24	29.03	8.06	4.83	4.83
	25-36	41.97	8.64	6.17	8.64
	Above 36	39.82	13.27	9.73	7.96
	P-value	0.002	0.069	0.139	0.195
Sheep	0-12	6.31	2.10	0	0
	13-24	12.85	5.0	3.57	4.28
	25-36	7.37	2.45	2.18	2.45
	Above 36	9.36	1.41	1.41	1.41
	P-value	0.208	0.105	0.098	0.040

Age of cattle/buffaloes in years while age of goats/sheep in months.

Table 5: Body weight based sero-prevalence of brucellosis in food animals (%)

Species	Body Weight (kg)	Test			
		RBPT	SAT	i-ELISA	c-ELISA
Cattle	100-300	6.97	4.65	3.87	3.87
	301-600	10.54	7.53	6.32	7.53
	Above 600	35.71	35.71	28.57	21.42
	P-value	0.000	0.001	0.003	0.002
Buffaloes	200-600	10	7.9	6.92	6.92
	601-1000	17.07	9.75	9.75	9.75
	P-value	0.137	0.602	0.464	0.464
Goat	10-30	19.23	6.15	3.84	4.61
	31-60	48.68	12.5	9.21	8.55
	P-value	0.000	0.066	0.067	0.182
Sheep	10-40	6.87	1.87	1.56	1.40
	41-80	10.96	2.40	1.95	2.40
	P-value	0.009	0.510	0.592	0.186

Table 6: Parity based sero-prevalence of brucellosis in food animals (%)

Species	Parity	Test			
		RBPT	SAT	i-ELISA	c-ELISA
Cattle	0-5	8.33	6.66	5.55	6.38
	6-10	19	12	10	10
	P-value	0.004	0.094	0.129	0.233
Buffaloes	0-5	12	8	6.4	7.2
	6-10	12.98	9.09	10.38	9.09
	P-value	0.837	0.787	0.314	0.631
Goats	0-3	33.48	5.04	3.21	2.75
	4-7	40	23.33	16.66	23.33
	P-value	0.485	0.002	0.008	0.000
Sheep	0-3	8.34	1.07	0.94	0.94
	4-7	8.11	2.17	1.98	2.17
	P-value	0.887	0.123	0.125	0.076

age (Bekele *et al.*, 2011) and prevalence was low in young stock than the adults (Sanogo *et al*., 2012). Age is known as one of the intrinsic factors influencing the sero-positivity of brucellosis (Megersa *et al.*, 2011). This influence can be explained by the fact that brucellosis is essentially a disease of the sexually mature animals, the predilection site being the reproductive tract, especially the gravid uterus (Abubakar *et al.*, 2012). So, susceptibility of an animal increases after sexual maturity in both sexes, because sex hormones and erythritol stimulate the growth of *Brucella* organism. Younger animals tend to be more resistant to *Brucella* infections; however, latent infections can occur in these animals (Gul *et al.*, 2013).

In relation to body weight, sero-prevalence of brucellosis in cattle with >600 kg body weight was higher than other groups. In goats and sheep, such difference was observed only through RBPT. This might be due to the fact that the body weight of mature animals was higher as compared to immature or younger calves, so the prevalence was higher in these animals.

A significant difference ($P<0.05$) was observed in sero-prevalence of brucellosis based upon parity through RBPT in cattle and in goats through SAT, i-ELISA and c-ELISA. Sero-prevalence based upon parity was higher in animals having more parity. It is due to the reason that prevalence of brucellosis increases with repeated exposure to parturition and other physiological stresses during gestation (Matope *et al.*, 2011; Hadush *et al.*, 2013).

Conclusion: Brucellosis is a highly contagious and zoonotic disease, resulting in heavy economic losses. The prevalence of the disease is associated with species, age, body weight and parity. Brucellosis is more prevalent in buffaloes as compared to the other food animals and also mature animals are at higher risk as compared to younger animals. However, it is not associated with the sex of the animal.

Acknowledgment: Financial support of the Higher Education Commission, Islamabad through Project # 20-1795/R&D, titled "Molecular Pathobiology of Brucellosis in Food Animals and Humans" is highly appreciated.

Author's contribution: STG and AK conceived the idea, designed the project, executed the experiment and analyzed the sera and data. All authors were involved in the interpretation of the data, write up and revision of the manuscript.

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