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RESEARCH ARTICLE

Epidemiology of Brucellosis at Different Livestock Farms in the Punjab, Pakistan

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ABSTRACT

Aim of the present study was to know the current prevalence of brucellosis at different Government and private Livestock Farms in Punjab, Pakistan. For this purpose, 2275 serum samples were collected and subjected to various diagnostic tests including Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT), i-ELISA and c-ELISA. Data thus collected was interpreted and subjected to Binary Logistic regression analysis to know the difference among different sources. Sensitivity and specificity values of these different tests were also determined. Seroprevalence of bovine brucellosis was significantly ($P<0.05$) higher in private livestock farms as compared to government farms through all the diagnostic tests applied. Sensitivity of SAT was higher in cattle (73.46%) as compared to the other species. c-ELISA was slightly more sensitive than i-ELISA. However, the specificity of all the tests in all species was 100%.

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INTRODUCTION

Brucellosis, is an important zoonotic disease, which causes considerable economic losses. Almost all domestic species can be affected. The disease is characterized by reproductive failure, particularly abortion in the last trimester of the gestation and placentitis in females and epididymitis, seminal vesiculitis and orchitis with sterility in males (Lopes *et al.,* 2010; Seleem *et al.,* 2010). Other manifestations of the disease include: still births, reduced milk yield, high frequency of retained placenta, prolonged calving interval, with excretion of the organisms in uterine discharges and milk and occasionally, hygroma and arthiritis are observed (England *et al.,* 2004; Abubakar *et al.,* 2012). A high incidence of temporary and permanent infertility could result in culling of animals. Some deaths may occur as a result of acute metritis following retained fetal membranes (Megid *et al.,* 2010).

Transmission typically occurs through contact with infected animals or materials with skin abrasions. The organism can be transmitted to humans through consumption of contaminated milk or dairy products prepared from unpasteurized milk such as soft cheeses, yoghurts, and ice-creams may contain high concentration of the bacteria, and direct contact with aborted fetus and fetal membranes, or sometimes through ingestion of meat from infected animals. Manure handling of infected animals is also a route of transmission to humans (Mantur and Amarnath, 2008; Christopher *et al.,* 2010).

A combination of serological and molecular methods are used for the diagnosis of brucellosis (Islam *et al.,* 2013). Isolation and identification of the causative organism remains a gold standard technique for diagnosis, but drawback is that it is time-consuming, hazardous and highly skilled personnel are required. For these reasons, Rose Bengal plate test is used as individual animal screening but there are chances of false positive, so further confirmation is needed. For this purpose serological tests like, serum agglutination test (SAT), Enzyme Linked Immunosorbent Assay (ELISA) are used (Poester *et al.,* 2010; Galińska and Zagórski, 2013).

In Pakistan, sero-prevalence of brucellosis has been reported (0-32.5%) in all livestock species on different farms and most of the times, it was RBPT and SAT based (Asif *et al.,* 2009). Further confirmation was dependant on a single confirmatory test, therefore, present study was planned and executed to investigate the sero-prevalence of brucellosis in these well-organized farms through basic serological reactions and also through ELISAs (i-ELISA and c-ELISA), and to find out the sensitivity and specificity of these confirmatory tests in relation to a screening test.

MATERIALS AND METHODS

Experimental animals: For this study, 2275 large (cattle and Buffaloes) and small animals (Goat and sheep) were selected randomly from different Government farms and Private farms located in Punjab, Pakistan. Government farms included in this study were Livestock Production Institute, Bahadurnagur, Okara; Livestock Experimental Station (LES), Qadirabad; LES, Khusab; LES, Allah Dad, Jahanian, Khanewal; LES, Khizerabad, Sargodha and Angora Goat Farm, Rakh Kharewala. Cattle samples from one well organized Private Farm, Faisalabad and Buffalo samples from a Private Farm, Tandlianwala were also collected. About 5ml blood without anticoagulant was collected from these animals and serum was separated by using standard procedure and stored at–20˚C till further analysis for sero-diagnosis.

Sero-diagnosis: Initially all the serum samples were screened through Rose Bengal plate test (RBPT) following the procedure described by Aldomy *et al.* (2013). RBPT positive samples were subjected to serum agglutination (Aldomy *et al.*, 2013), i-ELISA (*Brucella* I-ELISA Antibody Test, Kit #10-2700-10, Svanova, Sweden) and c-ELISA (*Brucella* Ab C-ELISA Test, Kit #10-2701-10) by following the procedures as described by the kit manufacturers.

Statistical analysis: Binary logistic regression analysis was applied through a statistical software MINITAB 16.0 version to know the difference in sero-prevalence at different livestock farms on the basis of all four diagnostic tests applied. Sensitivity and specificity of these confirmatory assays were calculated by following the statistical formulae described by Samad *et al.* (1994) as described below:

		Gold Standard Test		Total
		Positive	Negative	
Test to be compared	Positive	a	B	a + b
	Negative	c	D	c + d
	Total	a + c	b + d	a + b + c + d=N

To compare different assays following formulae indices are used; Sensitivity: a/a+c X 100; Specificity: b/b+d X 100; Overall agreement: a+d/N X 100.

RESULTS

Bovine brucellosis: Seroprevalence at different livestock farms: Sero-prevalence of brucellosis in cattle in relation to different sources was 8.82, 8.83 and 16.19% by RBPT in cattle from LPRI, Okara, LES, Qadirabad and Private farm, respectively. The difference in sero-prevalence among these three sources was statistically non-significant. Highest prevalence was recorded in private farm animals as compared to government cattle farms and the difference in sero-prevalence among government farm and private farm animals was statistically significant (P<0.05). Through RBPT lowest prevalence was recorded at LES, Qadirabad. The chances of sero-prevalence of brucellosis were 1.06 and 2.13 times higher at LPRI, Okara and private farm, respectively. Through SAT, sero-prevalence in animals from these three different sources was 4.20, 7.57 and 15.23% at LPRI, LES and Private Farm, respectively. Statistically, the difference in prevalence of brucellosis was significant (P<0.05). Highest prevalence was recorded at private farm followed by LES, Qadirabad and LPRI, Okara Farm. SAT results indicate that probability of disease prevalence at LPRI, Okara is 1.85 times lower and 2.19 times higher at private farm as compared to the LES, Qadirabad. Results of i-ELISA and c-ELISA also indicated higher prevalence at Private farm as compared to government farms and the difference was also statistically (P<0.05) significant. Odds ratio of i-ELISA based prevalence indicates that probability of the disease in comparison to LES, Qadirabad is 1.0 times and 4.57 times higher at LPRI, Okara and private farm, respectively. Sero-prevalence of brucellosis through c-ELISA have 2.79 times higher and 1.63 times lower probability at private farm and LPRI, Okara, respectively in comparison to LES, Qadirabad (Table 1).

Sero-prevalence of brucellosis at these three different sources was 4.49, 0 and 34.32% by RBPT in at LPRI, Okara, LES, Khusab and Private farm, respectively. The difference in sero-prevalence among these three sources was statistically significant. Highest prevalence was recorded in private farm animals as compared to government farms and the difference in sero-prevalence among government farm and private farm animals was also statistically significant (P<0.05). Through SAT, sero-prevalence in animals from these three different sources was 1.12, 0 and 25.37% at LPRI, LES and Private Farm, respectively. Statistically, the difference in prevalence of brucellosis was significant (P<0.05). Highest prevalence was recorded at private farm followed by LPRI, Okara Farm and none of the animals was positive at LES, Khusab. Results of i-ELISA and c-ELISA also indicate higher prevalence at Private farm as compared (Table 2).

Caprine and ovine brucellosis: Seroprevalence at livestock farms: Sero-prevalence of brucellosis in goats in relation to two different sources was 26.51 and 42.66% by RBPT at LES, Allah Dad and Angora Farm, respectively. The difference in sero-prevalence among these sources was statistically significant (P<0.05). Through SAT, sero-prevalence was higher (12%) at Angora Farm as compared to LES, Allah Dad, where prevalence was 6.81%. The sero-prevalence of brucellosis at LES, Allah Dad and Angora Farm was 6.06 and 7.33% through i-ELISA and it was 5.30 and 8% through c-ELISA, respectively. Logistic regression analysis indicated that the difference in sero-prevalence of caprine brucellosis at these two farms was non-significant by the SAT, i-ELISA and c-ELISA, however the chances of more positive animals were present at Angora Farm, Rakh kharewala (Table 3).

Sero-prevalence of ovine brucellosis was highest (13.36%) at Angora Farm, followed by LES, Khizerabad, where prevalence was 6.8%, and lowest (2.60%) was recorded at Allah Dad farm through RBPT. The difference in sero-prevalence among these three sources was statistically significant (P<0.05) and chances of ovine brucellosis were 2.72 and 5.76 time higher at LES, Khizerabad and Angora Farm, respectively as compared to Allah Dad farm. Through SAT, sero-prevalence was higher (2.25%) at Angora Farm as compared to Allah Dad

Table 1: Sero-prevalence of brucellosis in cattle at different Livestock farms

Parameters/Source	Total Animals	Positive	%	Coefficient	SE Co-ef	P Value	Odds Ratio
RBPT							
LES	132	11	8.83	-2.397	0.314	0.000	-
LPRI	238	21	8.82	0.062	0.389	0.872	1.06
Private	105	17	16.19	0.753	0.411	0.067	2.13
Chi-Squareuare	4.892						
P Value	0.087						
SAT							
LES	132	10	7.57	-2.501	0.328	0.000	-
LPRI	238	10	4.20	-0.625	0.461	0.175	0.54
Private	105	16	15.23	0.785	0.426	0.066	2.19
Chi-Squareuare	11.4407						
P Value	0.003						
i-ELISA							
LES	132	5	3.79	-3.234	0.455	0.000	-
LPRI	238	9	3.78	-0.001	0.568	0.998	1.00
Private	105	16	15.23	1.518	0.530	0.004	4.57
Chi-Squareuare	15.610						
P Value	0.001						
c-ELISA							
LES	132	8	6.06	-2.740	0.364	0.000	-
LPRI	238	9	3.78	-0.495	0.498	0.320	0.61
Private	105	16	15.24	1.024	0.454	0.024	2.79
Chi-Squareuare	13.267						
P Value	0.001						

Table 2: Sero-prevalence of brucellosis in Buffaloes at different Livestock farms

Parameters/Source	Total Animals	Positive	%	Coefficient	SE Co-ef	P Value	Odds Ratio
RBPT							
LES	56	0	0	-22.142	5211.16	0.997	-
LPRI	89	4	4.49	19.086	5211.16	0.997	1.94E+08
Private	67	23	34.32	21.493	5211.16	0.997	2.16E+09
Chi-Squareuare	17.6749						
P Value	0.000						
SAT							
LES	56	0	0	-22.518	6290.19	0.997	-
LPRI	89	1	1.12	18.041	6290.19	0.998	6844
Private	67	17	25.37	21.440	6290.19	0.997	2.04E+09
Chi-Squareuare	10.5945						
P Value	0.000						
i-ELISA							
LES	56	0	0	-22.579	6462.51	0.997	-
LPRI	89	1	1.12	18.095	6462.51	0.998	72244
Private	67	16	23.88	21.413	6462.51	0.997	1.992E+09
Chi-Squareuare	10.0687						
P Value	0.000						
c-ELISA							
LES	56	0	0	-22.579	6462.51	0.997	-
LPRI	89	1	1.12	18.095	6462.51	0.998	7244034
Private	67	16	23.88	21.413	6462.51	0.997	1.994E+09
Chi-Squareuare	10.0687						
P-Value	0.000						

and Khizerabad, where prevalence was 2.17 and 2%, respectively. The sero-prevalence of ovine brucellosis was 1.30, 1.6 and 2.08% through i-ELISA at Allah Dad, LES, Khizerabad and Angora Farm, respectively. Through c-ELISA, sero-prevalence was 1.30, 1.8 and 2.25 at Allah Dad, LES, Khizerabad and Angora Farm, respectively. But the difference in prevalence at these three farms was statistically non-significant through SAT, i-ELISA and c-ELISA, however probability of ovine brucellosis was higher at Angora Farm as compared to the other two farms (Table 4).

Sensitivity and specificity of diagnostic tests: The sensitivity and specificity of diagnostic tests used in this study were calculated considering RBPT as a standard test. The sensitivity of SAT was 73.46, 66.66, 27.27 and 23.93% in cattle, buffaloes, goats and sheep, respectively. The sensitivity of i-ELISA in cattle, buffaloes, goats and sheep was recorded as 61.22, 34.69, 19.19 and 19.65%, respectively. The sensitivity of c-ELISA was 67.34, 34.69, 19.19 and 21.36% was recorded in cattle, buffaloes, goats and sheep, respectively. The specificity of all three tests was 100% in all species when compared with RBPT (Table 5).

DISCUSSION

Brucellosis is a highly contagious bacterial disease which has not only zoonotic importance but also a disease of economic importance, 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; Abubakar *et al.*, 2012).

Highest prevalence of bovine brucellosis was recorded at private farms as compared to government livestock farms and the difference was statistically

Table 3: Sero-prevalence of caprine brucellosis at different Livestock farms

Parameters/Source	Total Animals	Positive	%	Coefficient	SE Co-ef	P Value	Odds Ratio
RBPT							
LES, Allah Dad	132	35	26.51	-1.019	0.197	0.000	-
Rakh Kharewala	150	64	42.66	0.723	0.257	0.005	2.06
P Value	0.004						
SAT							
LES, Allah Dad	132	9	6.81	-2.614	0.345	0.000	-
Rakh Kharewala	150	18	12	0.622	0.427	0.145	1.86
P Value	0.136						
i-ELISA							
LES, Allah Dad	132	8	6.06	-2.740	0.364	0.000	-
Rakh Kharewala	150	11	7.33	0.204	0.480	0.671	1.23
P Value	0.670						
c-ELISA							
LES, Allah Dad	132	7	5.30	-2.884	0.388	0.000	-
Rakh Kharewala	150	12	8	0.440	0.491	0.370	1.55
P Value	0.364						

Table 4: Sero-prevalence of ovine brucellosis at different Livestock farms

Parameters/Source	Total Animals	Positive	%	Coefficient	SE Co-ef	P Value	Odds Ratio
RBPT							
Allah Dad Farm	230	6	2.60	-3.619	0.413	0.000	-
Angora Farm	576	77	13.36	1.751	0.431	0.000	5.76
LES, Khizerabad	500	34	6.8	1.002	0.450	0.026	2.72
Chi-Square	24.6274						
P Value	0.000						
SAT							
Allah Dad Farm	230	5	2.17	-3.806	0.452	0.000	-
Angora Farm	576	13	2.25	0.038	0.532	0.943	1.04
LES, Khizerabad	500	10	2.0	-0.085	0.553	0.878	0.92
Chi-Square	0.08533						
P Value	0.958						
i-ELISA							
Allah Dad Farm	230	3	1.30	-4.326	0.581	0.000	-
Angora Farm	576	12	2.08	0.476	0.650	0.464	1.61
LES, Khizerabad	500	8	1.6	0.207	0.681	0.761	1.23
Chi-Square	0.689822						
P Value	0.702						
c-ELISA							
Allah Dad Farm	230	3	1.30	0.581		0.000	-
Angora Farm	576	13	2.25	0.645		0.387	1.75
LES, Khizerabad	500	9	1.8	0.671		0.626	1.39
Chi-Square	0.836612						
P Value	0.641						

significant and these results are in accordance to results observed by Naeem *et al.* (1990), Munir *et al.* (2011) and Poulsen *et al.* (2014), but contradicts to the results of Shafee *et al.* (2011), reported that prevalence of brucellosis was higher at government farms as compared to the private farms. Lower incidence of brucellosis at government farms is due to the reason that routine screening is carried out at these farms and infected animals are removed from the herd, so due to this the chances of brucellosis are decreasing day by day at government farms, while in commercial farms higher risk of brucellosis, most likely due to lack of herd health program, disorganized management system, adopted hygienic measures and frequent induction of high yielding animals without quarantine, so they certainly poses a zoonotic risk to the human population (Munir *et al.,* 2011; Sikder *et al.,* 2012).

Overall seroprevalence of caprine and ovine brucellosis in this study are lower as compared to previously reported in Pakistan and other parts of the world. Sero-prevalence of brucellosis in goats in relation to two different sources was higher at Angora Farm as compared to the LES, Allah Dad through all diagnostic tests. Sero-prevalence of brucellosis in sheep was highest at Angora farm, followed by LES, Khizerabad and lowest was recorded at LES, Allah Dad through all the diagnostic tests. This higher prevalence at Angora farm might be due to the prevalence of brucellosis in goats at that farm. According to previous findings, in sheep and goat brucellosis mixed and larger herd size also influence the prevalence of the disease, due to frequent contact with the infected animals, common feeding and watering points and poor management practices, which ultimately promotes the transmission of the disease (Al-Majali, 2005; Ali *et al*., 2009; Omer *et al.,* 2010; Bekele *et al.,* 2011; Asmare *et al.,* 2013; Teklue *et al.,* 2013).

Hence, no single serological test is appropriate in epidemiological situations, so the use of two or more diagnostic tests is usually recommended for maximum specificity and to eliminate the false positive cross-reactions. RBPT is a recommended screening test based on its cost, high sensitivity and easy to perform, particularly in endemic areas and its sensitivity is nearly 100% but specificity is 87.5% specificity. In this study RBPT was used as screening test and to eliminate false positive, SAT, i-ELISA and c-ELISA were applied and it was observed that sensitivity of these tests were lower than RBPT but specificity was 100% of all the three tests (Megersa *et al.,* 2011; Dürr *et al.* 2013; Andriopoulos *et al.,* 2015).

Table 5: Sensitivity and specificity of different diagnostic tests

Species	Test	Test +/-	RBPT		Sensitivity %	Specificity %	Overall agreement
			Positive	Negative			
Cattle	SAT	+	36	0	73.46	100	97.26
		-	13	426			
	i-ELISA	+	30	0	61.22	100	96.0
		-	19	426			
	c-ELISA	+	33	0	67.34	100	96.63
		-	16	426			
Buffaloes	SAT	+	18	0	66.66	100	95.75
		-	9	185			
	i-ELISA	+	17	0	34.69	100	95.28
		-	10	185			
	c-ELISA	+	17	0	34.69	100	95.28
		-	10	185			
Goats	SAT	+	27	0	27.27	100	74.46
		-	72	183			
	i-ELISA	+	19	0	19.19	100	71.63
		-	80	183			
	c-ELISA	+	19	0	19.19	100	71.63
		-	80	183			
Sheep	SAT	+	28	0	23.93	100	93.18
		-	89	1189			
	i-ELISA	+	23	0	19.65	100	92.80
		-	94	1189			
	c-ELISA	+	25	0	21.36	100	92.95
		-	92	1189			

Conclusion: Brucellosis prevalence in Pakistan is continuously increasing in private livestock farms as compared to government farms due to lack of awareness of these private farmers posing a threat to the human population which consume the milk of these farms. To control the disease training of private livestock farmers, field veterinarians, livestock assistant is essential to avoid future epidemics. RBPT can be used as a screening test, it is easy and economical and further confirmation can be done with either type of ELISA but c-ELISA is slightly more sensitive than i-ELISA

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Author's contribution: STG and AK designed the study. STG, MA and AS were involved in the execution of the project. STG and FR analyzed the data and wrote the manuscript. AK and IF interpreted the data and edited the manuscript. All authors approved the manuscript.

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