Seroprevalence of Bovine Brucellosis in Commercial Livestock Population of District Chakwal, Punjab

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Abstract

Brucellosis is recognized as an infectious zoonosis that has massive economic and public health consequences globally. The ailment is prevalent in livestock population of Pakistan including Punjab. A cross sectional study was conducted in order to elaborate the seroprevalence and risk factors of bovine brucellosis infection in commercial livestock population of district Chakwal, Pakistan. A total of 21 commercial livestock farms from where necessarily data and blood specimens of 12 animals were collected and 15 animals were sampled from one semen production unit. Blood specimens of 255 animals from 21 farms were analyzed with the aid of Rose Bengal Plate Test (RBPT) and Indirect Enzyme Linked Immunosorbant Assay (i-ELISA). In overall Seroprevalence of 7.45% was recorded for bovine brucellosis infection. Non-significant (p>0.05) difference was recorded between mean age of infected and non-infected animals. Highest

seropositivity was recorded for exotic cattle (25%) whereas that of lowest (1.53%) was recorded for local breed. Significant (p<0.05) association of infection was recorded with exotic breed, abortion history and first trimester of pregnancy with odds ratios of 31.64, 6.60 and 3.28 respectively. Similarly, highest risk ratios were also recorded for exotic breed (26.56), abortion history (5.30) and first trimester of pregnancy (2.89). Analysis of 255 blood specimens through RBPT revealed 8.63% seropositivity whereas that of 7.45% (n=19) when scrutinized through i-ELISA technique. Diagnostic accuracy of 92.55% was noted for RBPT. There is need to launch control programs against this notorious ailment bovine brucellosis to diminish its impact on human vigor and animal production.

Key Words

Seroprevalence, livestock, exotic cattle, bovine brucellosis, diagnostic accuracy

Introduction

Pakistan has agriculturally grounded economy where livestock sectors significantly subsidize in gross domestic product (GDP), being subsector of agricultural domain. The production potential of livestock is targeted by numerous infectious diseases. One of which is bovine brucellosis. Due to its economic, zoonotic and public health impact, the disease is categorized among most vital complications of livestock (Khan *et al*., 2020). Brucellosis is a zoonotic contagious disease caused by facultative, intercellular Gram-negative bacteria (coccobacillus) relative of genus Brucella (Nawaz *et al*., 2021). The associated clinical signs include abortions, hygroma, infertility, epididymitis, orchitis, reduced milk production and calf mortality. There are many relevant risk factors with prevalence of bovine brucellosis infection in diverse animals. The female animals were positive for brucellosis infection with abortion history or placenta maintenance (Saeed *et al.*, 2020). There are some more risk factors also linked with prevalence of pathogens. Age, gender, breed and body-composition score (Saeed *et al*., 2020). Herd level risk factors of bovine brucellosis include herd size, farm management practices, breeding methods (artificial/natural) or insemination techniques (Saeed *et al.*, 2020). In humans, the disease is characterized by, arthritis, joint pain, exhaustion, weakness which may lead to development of osteomyelitis, endocarditis, meningitis and death (Ducrotoy *et al*., 2017). Approximately five hundred thousand cases of infection are documented every year throughout the globe. In Pakistan, a Seroprevalence of 3.25 to 4.4% have been reported during 2019 (Saeed *et al.*, 2019, Ismail *et al*., 2018). Incidence of bovine brucellosis infection was highly noted worldwide. The occurrence of bovine brucellosis in

Pakistan was recorded very low 0 to 32.5% (Ismail *et al*., 2018). The overall prevalence of Brucella infection was reported 3.25-4.4% in different areas of Pakistan (Hussain *et al*., 2008). The prevalence 7.57% in cattle, 8.49% in buffaloes, 9.57% in goats and 2.14% in sheep were recorded respectively in different areas of Pakistan (Hussain *et al*., 2008). The infection is transmitted through aborted embryonic material, uterine release, milk and vaginal secretions (Jamil *et al*., 2020). The transmission of infection may also be carried out through semen by natural and artificial breeding methods (Arif *et al*., 2019). The humans who have direct contact with animals including animal handler, slaughter house workers, veterinarians, workers of different zoos, , livestock farmers, and laboratory personals are at greater risk of infection (Ndukum *et al.*, 2018). Bovine brucellosis infection may be diagnosed with the aid of various serological tests. Including Serum agglutination test (SAT), complement fixation test(CFT), polymerase chain reaction (PCR), Rose Bengal plate test (RBPT) and as well as Indirect enzyme linked immunosorbent assay (I-ELISA). Most commonly used serological tests for findings of brucellosis infection are ELISA and RBPT (Khan *et al*., 2020). RBPT is quick, economical and frequently used screening test for detection of brucellosis infection in remote zones. Agglutination technique which detects brucellosis antibodies in assumed serum samples. The test uses a suspension of *B. abortus* smooth cells stained with Rose Bengal dye (pink color) to identify *Brucella* agglutinins or clots (Garble *et al.*, 2017). i-ELISA is prominantly used for detection of brucellosis antibodies in serum. Due to high sensitivity, this is most widely used technique for detection of brucellosis infection in humans and animals (Ntivuguruzwa *et al.*, 2020). Utmost developed countries have been accomplished to eradicate bovine brucellosis. However, it is still endemic in most zones of globe (Franc *et al.*, 2018).

Purpose of research work/statement of novelty

Purpose of this research work was to estimate point prevalence of bovine brucellosis ailment in commercial livestock population all over the district, to highlight the risk factors associated with bovine brucellosis infection in commercial livestock population and to point out diagnostic accuracy of RBPT for detection of bovine brucellosis infection, by comparing with i-ELISA. At present, conferring to our knowledge, no published facts is available regarding Seroprevalence of bovine brucellosis infection in the study area projected.

Therefore, this study designed to estimate the seroprevalence of bovine brucellosis in district Chakwal to fill the knowledge gap for the current epidemiological status of the disease in the high-

cattle-farming zones of Punjab. Such information is dynamic and crucial in mapping and understanding the epidemiological distribution of the ailment, which can be used in the control of brucellosis in Chakwal and other districts in the region with comparable settings.

Material and methods

District Chakwal from Potohar region of North Punjab was selected as study area. The district has five administrative subdivisions or tehsils including Chakwal, Lawa Choa Saiden Shah, Kallar Kahar and Talagang. Total area of the district is 6609 square kilometers or 165443 acres which is comprised of hilly and plain areas. The district has 1083725 inhabitants, of which 12% are living in city areas. Due to its major involvement in national GDP (> 11%), livestock sector has been arisen as economic engine for poverty eradication in Pakistan. As compared to developed countries, major component (90%) of milk producing systems in Pakistan, is composed of market-oriented farming and maintenance small holdings and, followed by commercial dairy farming. Sample size for seroprevalence was measured by specifying the values of confidence level (95%), acceptable margin of error (5%), expected frequency (1%) and design effect (1%). The required sample size for estimation of seroprevalence was, n=15. Sample size for identification of risk factor was n= 212. A total of 12 animals were randomly choosed from each commercial dairy farm whereas 15 animals were selected from one semen production unit, for specimen and data collection. In this way 255 animals from 21farms were randomly selected for research. Base line information of each selected animal including age, gender, breed, specie, pregnancy status, pregnancy stage and abortion history were noted in pre designed Performa questionnaire. Sterile disposable syringe was used for collection of blood sample of animals from jugular vein with into the vacutainers with clot activator and EDTA accordingly.

Clotted blood specimens were centrifuged for 15 minutes at 5000 rpm (Saeed *et al*., 2019); serum was separated, transferred into serum cups and stored at -20℃ (Saeed *et al* ., 2019) tillfurther analysis. Serum samples were analyzed through RBPT, a rapid slide agglutination test for detection of Brucella antibodies. Samples exhibiting agglutination indicate the presence of specific Brucella antibodies and were therefore considered positive for brucellosis infection whereas those exhibiting no agglutination were considered as negative (Zakaria 2018).

All previously tested serum specimens were examined through ELISA using the Prio CHECK Brucella Ab 2 strip kit, catalog # 7610700, a five plated kit, with a capacity of 460 specimens, manufactured by Thermo Fisher Scientific. A sample was considered positive for brucellosis if it

tested positive on both the RBT and c-ELISA. Seroprevalence of brucellosis was assessed according to recommendations of Thurs field (2005) using following model.

$$\text{Seroprevalence} = \frac{\text{No of samples exhibiting positive outcome for brucellosis infection}}{\text{Total number of samples tested}} \times 10$$

Data was analyzed with the assistance of SPSS version 24 and Epi Info version 7.2.3.1 softwares. Frequency (%) was applied for categorical data and Mean ± S.D was used for quantitative data and independent sample t-test was applied for comparison of mean ± S.D for infected and non-infected groups. Chi-square test was practiced to compare the categorical data. Diagnostic accuracy was calculated by the aid of sensitivity, specificity, positive productive value and negative predictive value. To see the agreement between findings of BRBPT and ELISA, taking ELISA as gold standard, Kappa statistics was applied.

Results

Out of total 255 tested serum specimens, 19 (7.45%) displayed positive outcome for bovine brucellosis infection. Highest seropositivity was reported for female gender and exotic animals (Table 3.1). The mean age of examined livestock was statistically same in both positive (4.105 ± 1.93 years) and negative cases of bovine brucellosis (3.583 ± 1.57), *p*-value > 0.05. Exotic breed, abortion history and first trimester of pregnancy emerged as assumed risk factors of bovine brucellosis infection with significant ($p<0.05$) association / odds ratios of 31.64, 6.60 and 3.28 individually (Table 3.2). In the same way, highest risk ratios were also documented for exotic breed (26.56), abortion history (5.30) and first trimester of pregnancy (2.89). Analysis of data through conditional logistic regression also explained significant ($p<0.05$) association of infection with exotic breed and history of abortion.

Among baseline characteristics, breed and abortion history appeared as significant ($p<0.05$) exposure factors. Analysis of data through conditional logistic regression also explained significant ($p<0.05$) relation of infection with exotic breed and history of abortion (Table 3.3). Out of total 255 samples screened through RBPT, 22 (8.63%) exhibited positive outcome for bovine brucellosis infection although same samples revealed positive proportion of 19 (7.45%) when evaluated through ELISA technique. Application of Kappa test disclosed significant ($p<0.05$) difference between findings of RBPT and i-ELISA (Table 3.4).

Table 3.1: Percentage seroprevalence of bovine brucellosis in commercial livestock population of district Chakwal

Serial #	Variable	Total Animals n=255 Number (%)	Non Infected n=236 Number (92.54%)	Infected n=19 Number (7.45%)	Seroprevalence Overall (7.45%)
1	**Age**				
	Young (<2 years)	31(12.15%)	28(11.86%)	3(15.78%)	9.67%
	Adults (>2 years)	224(87.84%)	208 (88.13%)	16(84.21%)	7.14%
2	**Gender**				
	Male	60(23.53%)	59(25%)	1(5.26%)	1.66%
	Female	195(76.47%)	177(75%)	18(94.73%)	9.23%
3	**Specie**				
	Buffalo	4(1.56%)	4(1.69%)	0(0%)	00
	Cattle	191(78.03%)	173(73.3%)	18(94.73%)	9.42%
	Buff bull	19(7.45%)	19(8.05%)	0(0%)	00
	Cow bull	41(16.07%)	40(16.9%)	1(5.26%)	2.43%
4	**Breed**				
	Local	65(25.49%)	64(27.1%)	1(5.26%)	1.53%
	Cross bred	118(46.27%)	118(50%)	0(0%)	00

	Exotic	72(28.24%)	54(22.8%)	18(94.73%)	25%
5	**Pregnancy Status**				
	Pregnant	67(26.27%)	60(25.43%)	7(36.84%)	10.44%
	Non pregnant	128(50.20%)	117(49.5%)	11(57.89%)	8.59%
	NA(Male)	60(23.53%)	59(25%)	1(5.26%)	1.66%
6	**Pregnancy stage**				
	1^{st}-3^{rd} month	28(10.98%)	23(9.74%)	5(26.31%)	17.85%
	4^{th}-6^{th} month	22(8.62%)	21(8.89%)	1(5.26%)	4.54%
	$\geq 7^{th}$ month	17(6.66%)	16(6.77%)	1(5.26%)	5.88%
7	**Abortion History**				
	Yes	37(14.50%)	28(11.86%)	9(47.36%)	24.32
	No	158(61.96%)	149(63.13%)	9(47.36%)	5.69%
	NA (Male)	60(23.52%)	59(25%)	1(5.2%)	1.66%

Table 3.2: Odds based estimates risk factors of bovine brucellosis in commercial livestock population of district Chakwal

S/No	Variable/Exposure Factor	Cut Off Point	Infected	Non-infected	Odds Ratio	95% CI		Chi Square Value	P Value	
						Lower Limit	Upper Limit			
01	**Age**	Young (< 2 years)	3	28	1.39	0.3066	4.7466	0.2527	0.61551	
		Adult (≥ 2 years)	16	208	0.7190	0.2107	3.2621	0.2527	0.61551	
02	**Gender**	Male	1	59	0.1674	0.0078	0.9498	3.79	0.05149	
		Female	18	177	5.97	1.0528	128.29	3.79	0.05149	
03	**Specie**	Cattle	19	213	-1	0.59	-1	2.02	0.15	

									44	
		Buffalo	0	23	0.00	0.00	1.69	2.02	0.1544	
04	**Breed**	Local	1	151	0.0316	0.0015	0.1780	25.08	0.00000	
		Exotic	18	85	31.64	5.6167	676.93	25.08	0.00000	
05	**Pregnancy Status**	Pregnant	7	60	1.70	0.6061	4.5384	1.17	0.2775	
		Non-Pregnat	11	107	1.65	0.6356	4.4547	1.11	0.2919	
06	**Pregnancy Stage**	1^{st}-3^{rd} Month	5	23	3.28	0.9820	9.9774	4.92	0.02654	
		4^{th}-6^{th} Month	1	21	0.5698	0.0259	3.3913	0.2936	0.5879	
		≥7 month	1	16	0.7646	0.0343	4.6618	0.0647	0.7991	
0	**Abortion**	Yes	9	28	6.60	2.4021	18.0559	17.79	0.00	

7	**History**								00	
		No	9	149	0.52	0.19 96	1.3752	1.84	0. 17 40	

Table3.3: Risk factors of bovine brucellosis infection (exotic breed and abortion history) in commercial livestock population of district Chakwal

Variable/Exposure Factor		**β**	**S.E.**	**Wald**	**df**	**P-value**	**Odds Ratio (OR)**	**95.0% Confidence Interval**	
								Lower	**Upper**
Step 1[a]	***Exotic Breed***	4.105	1.039	15.612	1	<0.001	60.667	7.916	464.909
	Constant	-5.204	1.003	26.934	1	<0.001	.005		
Step 2[b]	***Exotic Breed***	4.033	1.048	14.821	1	<0.001	56.438	7.241	439.859
	Abortion History (Yes)	1.743	.589	8.749	1	.003	5.715	1.800	18.138
	Constant	-5.631	1.035	29.605	1	<0.001	.004		

Table 3.4: Diagnostic accuracy of RBPT to detect bovine brucellosis

Parameter	**Estimate**	**Lower - Upper 95% CIs**
Sensitivity	57.89%	(36.28, 76.86)
Specificity	95.34%	(91.85, 97.38)
Positive Predictive Value	50%	(30.72, 69.28)

Negative Predictive Value	96.57%	(93.37, 98.25)
Diagnostic Accuracy	92.55%	(88.66, 95.18)

Discussion

Brucellosis is a zoonotic contagious disease caused by facultative, intercellular Gram-negative bacteria (cocobacilus) belongs to genus Brucella (Ayoola *et al*., 2017, Nawaz *et al*., 2021). Due to its zoonotic, economic and public health impact brucellosis is included among most important problem of livestock (Khan *et al*., 2020). Seroprevalence of bovine brucellosis in cattle and buffaloes was compareable with Ismail *et al* (2018), Nasir*et al* (2004) and by Husain *et al* (2008). The prevalence of bovine brucellosis infection in different animal of selected districts of Pakistan was studied by Saeed *et al* (2019). The overall Seroprevalence 3.2% recorded. In present findings the overall Seroprevalence was 7.45% recorded which is higher than Saeed *et al* (2019) findings. Seroprevalence of brucellosis in cattle and buffaloes investigated among livestock farms in Punjab, Pakistan. Overall Seroprevalence of brucellosis infection by RBPT and i-ELISA 3.9% and 3.3% were recorded by Jamil *et al* (2020). This study showed the less seropositivity for brucellosis then current studies 7.45% was recorded. Seropositivity for brucellosis infection in cows and buffaloes was estimated by RBPT and i-ELISA. The apparent geographical variation in the seroprevalence might reflect differences in the levels of natural immunity. In addition, sensitivities and specificities of the diagnostic methods used among researchers might also affect the outcome.

Risk factors related with bovine brucellosis in individual level and herd level of seven districts were considered by Arif *et al* (2019). More seropositivity was documented in herds with history of last trimester abortion with OR=2.06 as compared to herd without abortion record. Herd size was also found the risk factor the herd with five to eight buffaloes OR=3.80 more than eight with OR=3.80 were more seropositive than those with less buffaloes in groups. In recent studies abortion history, exotic breed, and first trimester of pregnancy emerged as risk factors of bovine brucellosis infection with OR=31.64, 6.60 and 3.28 correspondingly. In combined findings, abortion history was the shared risk factor for bovine brucellosisailment. Associated risk factors with bovine brucellosis infection in livestock wild life urban and periurban areas of Rwanda were explored by Nitvuguruzwa *et al* (2020). Significant association of zoonosis was recorded with ≥ 5 years age OR=3.0, mixed herds of small ruminants and cattle, previous abortion history OR=2.5

cattle rearing near wild life and everyday replacement of livestock. In present investigations the exotic breed, abortion history and first trimester of pregnancy popped out as menace factors of bovine brucellosis infection with OR= 31.64, 6.60 and 3.28 correspondingly. In previous findings of Nitvuguruzwa *et al* (2020) abortion history with OR=2.5 and current studies abortion history with OR=6.60 was found. In existing studies the abortion history was seemed as high risk factor. Seroprevalence of *Brucella abortus* in dairy animals of Pothohar region of Pakistan was considered by Ali *et al* (2013). The exotic breed with OR=2.0 was showed as risk factor. In present findings the exotic breed with OR=31.64 appeared as risk factor. RBPT and ELISA tests for diagnosis of brucellosis infection in cattle of organized herd were compared by Kushawaha *et al* (2016) showing seropositivity by RBPT 32.61% and by ELISA 33.85%, Gurbiklek *et al* (2017) showing seropositivity of RBPT and ELISA 81.3% and 83.7% respectively and Jamil *et al* (2020) showing seropositivity RBPT and ELISA 3.9% and 3.3%. these findings have shown very close results. In present findings RBPT 8.63% and 7.45% by ELISA seropositivity was recorded. In current studies RBPT and ELISA showed very close results. It showed that the diagnostic accuracy of RBPT is very high.

Serological tests RBPT and SAT was investigated by Nasir *et al* (2004). The overall findings by RBPT were 17.20% whereas on SAT 8.0% recorded. In current studies seropositivity in buffaloes and cows by RBPT 8.63% and 7.45% by ELISA recorded. The RBPT specificity recorded high in current findings but in Nasir *et al* (2004) findings less specificity was noted. So RBPT diagnostic accuracy is very high and very near to ELISA. The seropositivity for bovine brucellosis infection by RBPT was 12.53% whereas by ELISA prevalence 2.39% recorded was investigated by Khan *et al* (2020). This study showed the low sensitivity of RBPT and high sensitivity for ELISA. In present research the sensitivity of RBPT and ELISA were very close.

Sensitivity and specificity of ELISA and RBPT for diagnosis of brucellosis infection was investigated by Zakria (2018) showing RBPT exhibited 79.12% sensitivity, 39.58% specificity and 70.87% accuracy when compared with PCR whereas ELISA exhibited 55.49% sensitivity, 52.08% specificity and 54.78% accuracy. In current studiesthe RBPT showed 57.89% sensitivity, 95.34% specificity and 92.55% diagnostic accuracy. The sensitivity and specificity of three diagnostic tests RBPT, i-ELISA and CFT for diagnosis of brucellosis infection were explored by Getachew *et al* (2016). 89.6% sensitivity, 84.5% specificity assessed for RBPT.96.8% sensitivity, 96.3% specificity recorded for ELISA. In current studies sensitivity 57.89%, specificity 95.34%

of RBPT valued. Getachew *et al* (2016) outcomes showed high sensitivity but low specificity as compared to present research outcomes.

1. Conclusion

Bovine brucellosis is notorious disease of being public health significance and regular herd based with many risk factors is penetrating in livestock populations globally including Pakistan. Exotic animals are in front of higher risk of infection as compared to local and hybrid animals. More studies should be planned to investigate the reason. Due to its rapidity, low cost and simple protocol, RBPT, although, but findings of the present-day study suggest that screening of infection and confirmation of positive cases may be carried out through RBPT along with standard ELISA test. Multisectoral One Health surveillance and control approaches are in need of development and implementation to minimize the ailment burden in animals as well as in humans.

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