Eco. Env. & Cons. 26 (November Suppl. Issue) : 2020; pp. (S300-S306)
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ISSN 0971–765X

Sero-prevalence and hematological investigation of *Bovine brucellosis* under extreme ecological conditions

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(Received 20 April, 2020; Accepted 30 June, 2020)

ABSTRACT

This study evaluated the prevalence of brucellosis in bovines under extreme ecological conditions. Rose Bengal Plate Agglutination Test (RBPT) and Serum Agglutination Test (SAT) and hematology used to study the prevalence of *Brucella*. A total of 949 bovines (n=234 buffaloes; n=715 cattle) tested. The results of RBPT showed 31 animals positive for Brucella. RBPT positive samples subjected to SAT, and only ten were found positive. Cattle and buffaloes found positive with RBPT showed a significant decrease in the values of Haemoglobin (Hb) and lymphocytes. Significant decrease in PCV, TEC, lymphocytes, and an increase in monocytes observed in cattle only. Chi-square test and Student's *t*-test and service solutions (SPSS) used for statistical analysis. The overall seroprevalence of brucellosis was found to be 3.27% and 32.26% by RBPT and SAT, respectively. RBPT was found more sensitive, and less specific as compared to SAT. Area-wise prevalence of brucellosis indicated significantly difference among the different areas. Moreover, higher prevalence of brucellosis observed in buffaloes than in cattle. Hematological abnormalities also observed in infected animals. This research describes changes in hematological parameters both in brucellosis positive cattle and buffaloes at Livestock forms and small domestic herds under extreme ecological conditions. More work is required to elucidate the impact of brucellosis on serum chemistry, immune-modulatory features and hormonal profile in cattle and buffaloes.

Key words: *Bovine Brucellosis, Seroepidemiologic Studies, Livestock.*

Introduction

Brucellosis characterized as the second important infectious as well as zoonotic disease after rabies and present around the globe (Cutler and Whatmore, 2003; Lapaque *et al.*, 2005) known to be highly contagious, economically important, zoonotic bacterial diseases in animals (OIE, 2000). Members of genus *Brucella* persist and replicate within the host cell leading to brucellosis (Ficht, 2003). Genus encompasses species named *Brucella*, *B.abortus* in cattle, *B. melitensis* in goats, *B. suis* in swine and *B. ovis* sheep (Radostits *et al.*, 2000) leading to decreased milk production, weak offspring, abortion, weight loss, lameness, infertility and death of infected animals (Radostits *et al.*, 2000; Soomro *et*

al., 2014). Brucellosis is specifically a disease of sexually mature animals. Localization and growth of the virulent strains of *B. abortus* stimulated by Erythritol (Smith *et al.,* 1962). Contact with vaginal discharge, infected placenta, fetus, fetal membranes, and fluids helps in disease transmission. Consumption of contaminated milk is a major source of zoonosis (Sheikh *et al.,* 1967). Brucellosis effect the livestock handlers and Veterinary professionals as well (Khan *et al.,* 2018). In human, occupational cases have most frequently observed which involve butchers, veterinarians, and even farmers. Incidences and prevalence of Brucellosis have been observed to be varying region to region (Radostits *et al.,* 2000). In different areas of Pakistan, the seroprevalence of brucellosis has recorded as lower as 0.33% to 0.65% (Sheikh *et al.,* 1967) and as higher as up to 21% to 26% (Sharma and Adlakha, 1997). Rose bengal plate agglutination test (RBPT) and enzyme-linked immune sorbent assay (ELISA) are major serological tests used for the diagnosis of brucellosis (Gul and Khan, 2007).

In Pakistan, very limited research work is available on variations in hematological parameters during brucellosis in cattle and buffaloes. Therefore, a study was planned to determine significant diagnostic variations in the hematological parameters of cattle and buffaloes suffering from brucellosis in Livestock farms and small domestic herds of Thal desert of (district Bhakkar), Punjab, Pakistan. These farms supply milk and meat to the surrounding areas of Bhakkar and Mianwali districts leading to a high incidence of infection in animal and human of these districts. The main objective of the study was to investigate the prevalence and effects of brucellosis on hematological parameters in livestock at different Livestock Farms and small domestic herds of Thal desert in (district Bhakkar) Punjab Pakistan and make species to species and area to area comparison.

Materials and Methods

Universe of the Study

The study conducted in district Bhakkar found at 31.8621° N, 71.3824° E Desert Thal Punjab Pakistan. Desert Thal is the third largest desert in Pakistan, situated in the almost center to the west of Province Punjab (Fig. 1); it covers an estimated area of 20,000 km.It is boarded by Indus and Jehlum Rivers on western and eastern flanks respectively. District Bhakkar has four tehsils, named Menkaira, Daryakhan, Kallurkoat, and Tehsil Bhakkar.The study was carried out in (i) Union Council Ghulaman area in Tehsil Kallurkoat (ii) Union Council 205/TDA area in Tehsil Bhakkar, (iii) Union Council MalanaDagar area in Tehsil Kallurkoat (iv) Union Council RakhMahni area in Tehsil Menkaira**.** All the information collected on a pre-designed questionnaire and experiments conducted at District Diagnostic Laboratory for Livestock, Mianwali and Bhakkar. A total of 949 adult female bovines (cattle=715; buffalo=234) were selected randomly in the study area. After proper restraining, 5 mL blood collected from the jugular veins in disposable needle syringes. The blood was allowed to clot at room temperature (25 °C) for four hours; afterward, the sera separated in screw-capped sterilized tubes. The blood samples, from which serum not gathered easily, were subjected to centrifugation at 1500g for 10 minutes. All the serum samples kept under refrigeration (-20 °C) until further use.

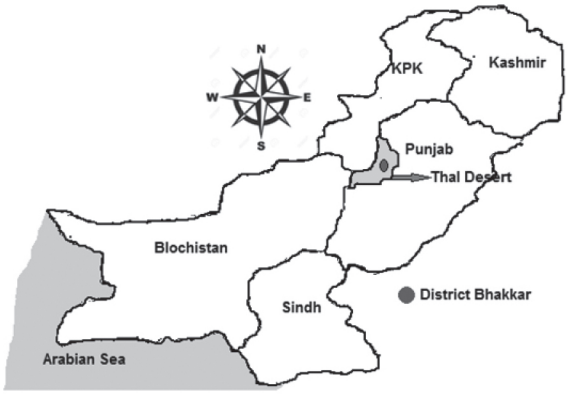

Fig. 1. Map of Pakistan Showing Thai Desert. Area of sampling is highlighted

Rose Bengal Plate Agglutination Test (RBPT)

The presence of *Brucella* antigen was investigated (OIE, 2013) using RBPT. Briefly, antigen and sera were allowed to reach room temperature (22±4 °C). Around 25-30 µL of serum from each sample was placed on a glass slide and the same volume (25-30ul) of antigen spotted near the serum sample. Afterward, serum and antigen were mixed thoroughly using a micropipette tip for each test and kept at room temperature for about 4 minutes. To verify the sensitivity of test a control serum used with every tested serum.

Serum Agglutination Test (SAT)

The serum samples positive after RBPT were reconfirmed using SAT (Stemshorn *et al.,* 1985). Briefly, five conical agglutination tubes placed in the rack for each test serum sample. 0.8 mL of normal saline solution containing 0.5% phenol added to the first tube and 0.5ml in the remaining four. 0.2 mL of test sera was added to the first tube and mixed thoroughly; this would make 1/5 dilution. 0.5 mL was withdrawn from the first tube and transferred into the second tube. After mixing well, 0.5 mL was carried to the 3rd and so on up to the fifth tube where after mixing, 0.5 mL discarded. Now the dilution in each tube would be 1/5, 1/10, 1/20, 1/40 and 1/80 respectively. 0.5 mL of the standardized *B. abortus* concentrate antigen (diluted 1:10) was added to each tube, containing serum dilution, giving a series of final dilutions from 1/10, 1/20, 1/40, 1/80 and 1/160 respectively. The rack then incubated at 37 °C for 18 to 20 hours. Both known positive and negative sera kept and controlled. Results observed based on clearing of the suspension along with clumping of the organisms and permanency of the sediments upon gentle shaking.

Hematological Examination

In order to investigate the changes in the blood picture of brucellosis-infected animals, hematological analyzer Sysmex XP-100 used. A total of 40 bovines (cattle=20; buffalo=20) comprising of 20 positive animals (cattle=10; buffalo=10) for RBPT and 20 negative animals (cattle=10; buffalo=10) for brucellosis were selected. Through jugular vein puncture, 5ml of blood was collected in sterile tubes coated with anticoagulant (EDTA @1 mg/mL).

Statistical Analysis

All the data was managed and analyzed by using Non-parametric, Chi-square test whereas data on hematology were analyzed by Student's *t*-test using statistical product and service solutions (SPSS) 16.0 for Windows. Probability (p) value <0.05 was considered significant.

Results and Discussion

Effects of Brucellosis on Hematological Parameters

The value of erythrocyte sedimentation rate (ESR) was found unchanged during the first hour in Brucella positive and negative buffaloes and cattle. A significant ($p \leq 0.05$) decrease in the hemoglobin count of positive brucellosis buffaloes (10.95±0.90 g/dL) observed than in negative ones (13.67±0.65 g/dL). Hemoglobin count was found to be significantly decreased ($p \leq 0.05$) in brucellosis positive cattle (6.79±2.94 g/dL) than in the negative (9.29±0.53g/dL). Packed cell volume (PCV) was insignificantly ($p \geq 0.05$) decreased in brucellosis positive buffaloes (29.67±5.41%) than negative ones (31.93±5.43%). Packed cell volume was significantly ($p \leq 0.05$) decreased in brucellosis positive cattle (25.11±5.55%) than negative ones (35.05±1.70%). Total erythrocyte count (TEC) showed slightly insignificant ($p \geq 0.05$) decrease in brucellosis positive buffaloes ($5.43 \pm 1.64 \times 10^6/\mu L$) than negative ones ($6.13 \pm 1.54 \times 10^6/\mu L$). Total erythrocyte count was significantly ($p \geq 0.05$) decreased in brucellosis positive cattle ($6.63 \pm 2.04 \times 10^6/$ìL) than negative ones ($8.59 \pm 1.02 \times 10^6/\mu L$). Total leucocyte count (TLC) was slightly insignificant (pe"0.05) decrease in brucellosis positive buffaloes ($8.15 \pm 0.70 \times 10^3/\mu L$) than negative ones ($8.45 \pm 2.62 \times 10^3/\mu L$). TLC was slightly insignificant ($p \geq 0.05$) decreased in brucellosis positive cattle ($5.53 \pm 3.87 \times 10^3/\mu L$) than negative ones ($7.4 \pm 2.64 \times 10^3/\mu L$). In order to study differential leukocyte count (DLC), basophils, eosinophils, lymphocytes, monocytes, and neutrophils observed. Amount of basophils was slightly insignificant ($p \geq 0.05$) decrease in brucellosis positive buffaloes (0.16±0.23%) than negative ones (0.22±0.28%). Amount of basophils was slightly insignificant ($p \geq 0.05$) decrease in brucellosis positive cattle (0.87±0.43%) than negative ones (1±0.46%). Amount of eosinophils was slightly insignificant ($p \geq 0.05$) increased in brucellosis positive buffaloes (4.82±2.87%) than negative ones (3.16±2.99%). Amount of eosinophils was slightly insignificant ($p \geq 0.05$) increased in brucellosis positive cattle (6.55±1.47%) than negative ones (5.71±2.5%). Amount of lymphocytes was decreased significantly ($p \leq 0.05$) in positive brucellosis buffaloes (32.46±5.54%) than negative ones (37.75±5.57%). Amount of lymphocytes was decreased significantly ($p \geq 0.05$) in brucellosis positive cattle (37.75±5.57%) than negative ones (62.28±5.96%). Amount of monocytes was slightly insignificant ($p \geq 0.05$) increased in brucellosis positive buffaloes (2.79±1.28%) than negative ones (1.66±1.42%). Amount of monocytes was significantly ($p \geq 0.05$) increased in brucellosis positive cattle (5.97±1.53%) than negative ones (3.65±0.58%). Amount of neutro-

phils was slightly insignificant (p≥0.05) decreased in brucellosis positive buffaloes (40.70±5.70%) than negative ones (41.61±6.17%). Amount of neutrophils was slightly insignificant (p≥0.05) decreased in brucellosis positive cattle (27.73±5.39%) than negative ones (28.49±6.83%). All the data presented in Table 3.

In the present findings, (Table 1, Figure 2, 3) RBPT detected more positive samples of brucellosis as compared to SAT. Similar results were obtained in previous study by Nasir and Ikram-ul-Haq (2005) in which serum samples collected from buffaloes and cattle of different livestock farms of Punjab, Pakistan and domestic herds and applied RBPT for *Brucella* antigen. Present results of brucellosis prevalence by RBPT in cattle and buffaloes showed that *Brucella* was more prevalent in buffaloes than cattle (Table 2, Figure f). High seroprevellance of *Brucella* antibodies also reported in buffaloes as compared to cattle in government livestock farms of Punjab, privately owned farms and Gawala colony of Lahore (Ismail *et al.,* 2018; Munir *et al.,* 2011). Similar results were obtained in Rahman *et al.* (2011) study, in which used RBPT and ELISA as screening and confirmatory tests respectively. Meanwhile, Ghodasara and Bhanderi (2010) determined the high prevalence of brucellosis by RBPT in cattle as compared to buffaloes and found RBPT and SAT methods more useful in the nationwide survey.

Hemoglobin value in the current study was observed lower than the reference value. This result was similar to the findings done of previous studies, in on old women, camel, and cattle correspondingly (El-Boshy *et al.,* 2009; Gürkan *et al.,* 2003; Kushwaha *et al.,* 2014). Intracellular position of Brucella spp. Might be a cause of hemoglobin reduction (Sikder *et al.,* 2012). It could be attributed to the elevated blood levels of inflammatory chemical mediators as IL-1â back non-regenerative anemia asso-

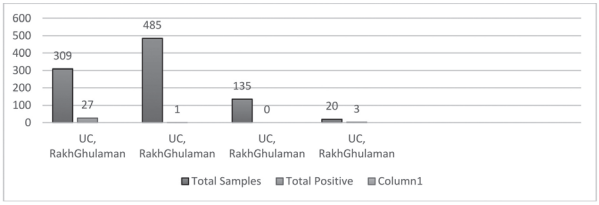

Fig. 2. U.C wise Prevallance of Brucella with RBP

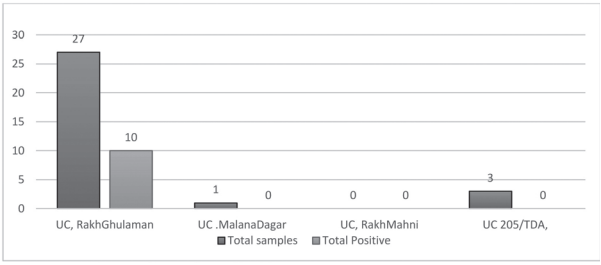

Fig. 3. U.C wise Prevallance of Brucella with SAT

Table 1. Uc-wise prevalence of brucellosis in cattle and buffaloes of different Livestock Farms & Domestic Herds as tested by RBPT and SAT

Livestock Farms & Domestic Herds of the Area	RBPT				SAT			
	N	n	%	p-value	N	n	%	p-value
UC, RakhGhulaman	309	27	8.74	0.000*	27	10	37.4	0.335
UC .MalanaDagar	485	1	0.21		1	0	0	
UC, RakhMahni	135	0	0		0	0	0	
UC 205/TDA,	20	3	15		3	0	0	
Total	949	31	3.27		31	10	32.26	

UC = Union Council N=No. of sampled animals, n=No. of positive animals, %=Percentage, *Significant (p≤0.05)

Table 2. Specie-wise Prevalence of Brucellosis in Buffaloes and Cattle of Different Livestock Farms & Domestic Herds tested by RBPT and SAT

Species	RBPT				SAT			
	N	n	%	p-value	N	n	%	p-value
Buffalo	234	12	5.13	0.065	12	8	66.67	0.001*
Cattle	715	19	2.66		19	2	10.53	
Total	949	31	3.27		31	10	32.26	

N=No. of sampled animals, n=No. of positive animals, %=Percentage, *Significant (p ≤ 0.05)

ciated with chronic disease 9220 (Dinarello, 2005).

These results indicate that PCV value alters by brucellosis positivity in El-Boshy *et al.* (2009) study recorded the same findings in brucellosis positive and negative camels.TEC value showed a reduction than the reference value. These findings were verified in which noted little (6.41 to 6.44%) reduction of TEC value in brucellosis positive camel and cattle respectively (El-Boshy *et al.*, 2009; Kushwaha *et al.*, 2014). The decrease in TLC found in agreement with those recorded in (El-Boshy *et al.*, 2009; Kushwaha *et al.*, 2014). Basophil count was found close with the

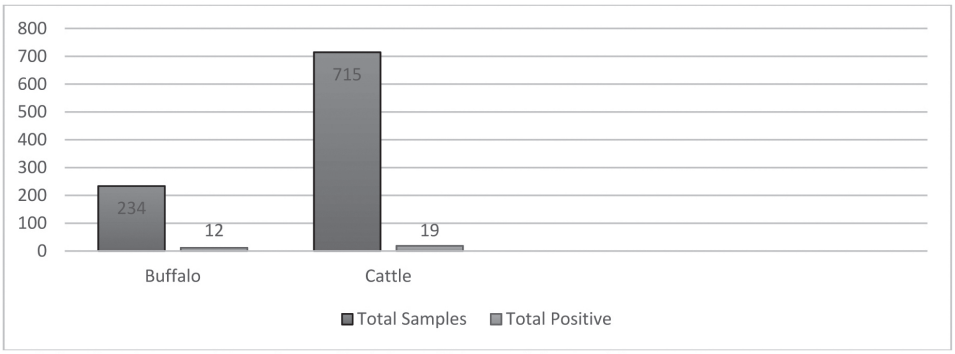

Fig. 4. Specie-wise Prevalence of Brucellosis in Buffaloes and Cattle with RBPT

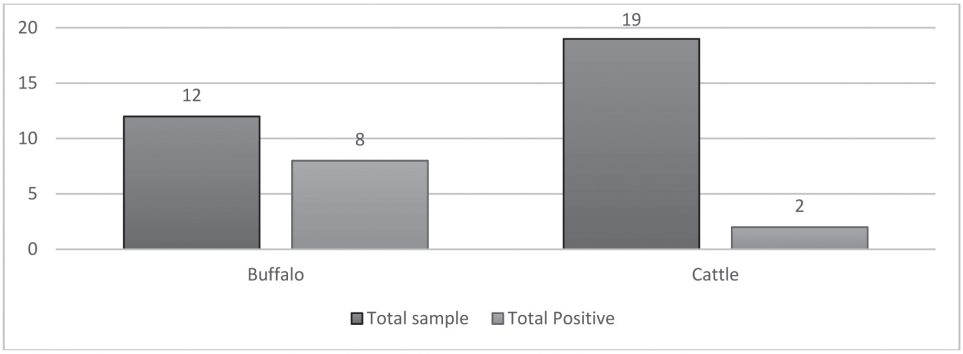

Fig. 5. Specie-wise Prevalence of Brucellosis in Buffaloes and Cattle with SAT

Table 3. Hematological examination of brucellosis positive and negative buffaloes and cattle of different Livestock Farms and Domestic Herds

Haematological parameters	Buffalo			Cattle		
	Brucellosis positive (n = 10) Mean ± SD	Brucellosis negative (n = 10) Mean ± SD	p-value	Brucellosis positive (n = 10) Mean ± SD	Brucellosis negative (n = 10) Mean ± SD	p-value
ESR (mm in firsthour)	0	0	-	0	0	-
Hb (g/dL)	10.95 ± 0.90	13.67 ± 0.65	.000	6.79 ± 2.94	9.29 ± 0.53	.024
PCV (%)	29.67 ± 5.41	31.93 ± 5.43	.364	25.11 ± 5.55	35.05 ± 1.70	.000
TEC (10^6/µL)	5.43 ± 1.64	6.13 ± 1.54	.338	6.63 ± 2.04	8.59 ± 1.02	.014
TLC (10^3/µL)	8.15 ± .70	8.45 ± 2.62	.734	5.53 ± 3.87	7.4 ± 2.64	.223
Basophils (%)	0.16 ± 0.23	0.22 ± 0.28	.607	0.87 ± 0.43	1 ± 0.46	.522
Eosinophils (%)	4.82 ± 2.87	3.16 ± 2.99	.221	6.55 ± 1.47	5.71 ± 2.5	.372
Lymphocytes (%)	32.46 ± 5.54	37.75 ± 5.57	.047	44.65 ± 11.39	62.28 ± 5.96	.000
Monocytes (%)	2.79 ± 1.28	1.66 ± 1.42	.078	5.97 ± 1.53	3.65 ± 0.58	.001
Neutrophils (%)	40.70 ± 5.70	41.61 ± 6.17	.736	27.73 ± 5.39	28.49 ± 6.83	.786

ESR = Erythrocyte sedimentation rate, Hb = Hemoglobin, PCV = Packed cell volume, TEC = Total erythrocyte count, TLC = Total leukocyte count, DLC = Differential leukocyte count

results of the study in camels and cattle (El-Boshy *et al.,* 2009; Sikder *et al.,* 2012). Unlikely higher basophil values were reported by Forbes (1996) in moose. Forbes (1996) who worked on brucellosis positive moose and in cattle (Sikder *et al.,* 2012) also showed increased eosinophil percentage accordingly. Lymphocytes percentage was similar which reported lower lymphocytes value in camel, cattle and human patients respectively (El-Boshy *et al.,* 2009; Erbay *et al.,* 2009; Kushwaha *et al.,* 2014). A study carried out on lymphadenitis of camels which were infected experimentally with *B. abortus* (Damir *et al.,* 1989). Similarly, after intraperitoneal inoculation of strains of *B. abortus* in mice, it is observed a depletion of lymphoid tissue in the white pulp (Palmer *et al.,* 1996; Stevens *et al.,* 1994). Monocytes percentage recorded above observed close to the findings of Forbes *et al.* (1996) study, in which study carried out on brucellosis positive moose, in cattle (Sikder *et al.,* 2012), in camels (El-Boshy *et al.,* 2009). Radostis (2000) suggested that monocytes increase in bacterial infections especially which are non-specific. Similarly, when bovine fetus infected naturally or experimentally with brucellosis (Enright *et al.,* 1984), lymphoid depletion in the cortex region of the thymus was observed which gave lymphopenic condition. These results were also inconsistent with that of observed (El-Boshy *et al.,* 2009; Kushwaha *et al.,* 2014; Sikder *et al.,* 2012).

Conclusion

The current research shows RBPT & SAT has more effective serological tests. These tests could employ for screening of Brucellosis. RBPT can be employed as a screening test for dairy herd while the SAT used as a validation test and culling of the animal should base on SAT. As a zoonotic disease, it is very much important to develop strategies for the control of the disease in animals especially in common food-producing animals like cattle and buffaloes. Milk from infected livestock, especially unpasteurized milk, is a potential source of brucellosis infection for humans which may, in turn, cause health complications. Individuals in Pakistan are especially at a higher risk of acquiring the infection. Therefore brucellosis control programs should be initiated using isolation and testing of Brucella for effective control of this disease in Pakistan as a whole.

Acknowledgement

This study would like to give high gratitute to the involved institution which are Office of Assistant Disease Investigation Officer Bhakkar Pakistan, Disease Diagnostic Laboratory Mianwali Pakistan, State Key Laboratory of Agricultural Microbiology, College of Veterinary Medicine, Huazhong Agricultural University, China, Institute of Food Science & Nutrition, University of Sargodha, Pakistan and Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia for giving permission and support during this research process

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