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Babesia bovis in Large Ruminants in Pakistan - Molecular Detection and Haemato-Biochemical Alterations

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ABSTRACT

***Background*:** Babesiosis is endemic in Pakistan and is one of the most important bovine diseases that causes huge economic losses and high mortality in young animals. A hematobiochemical study was conducted to unveil the difference between diseased and healthy animals in selected districts i.e., Faisalabad (31° 25' 7.3740'' N and 73° 4' 44.7924'' E), Toba Tek Singh (30° 58' 9.7392'' N and 72° 27' 40.7484'' E) and Jhang (31° 16' 40.9656'' N and 72° 18' 42.3360'' E) of Punjab, Pakistan. ***Materials, Methods & Results*:** A total of 518 (Cattle = 360, Buffalo = 158) blood samples were collected. The samples were analyzed by polymerase chain reaction (PCR) targeting apocytochrome b-gene (*Babesia bovis*-gene) (CYTb) followed by haemato-biochemical analysis. Chi-square test for univariate analysis was used to analyze the data. In summer the PCR-based prevalence was 29.4 (53/180) and 24.05% (19/79) in cows and buffaloes, respectively. On the other hand, in winter results showed that 12.7 (23/180), 13.92 % (11/79) samples positive for Babesia genus from cows and buffaloes, respectively. The positive samples were further investigated for hematological and biochemical analysis. The results revealed that, the mean value of hematological parameters like RBCs, Hb, PCV, MCV and MCHC was significantly ($P < 0.05$) decreased in infected animals (cows and buffaloes) as compared to the non-infected ones. While the biochemical parameters like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), cholesterol and Lactate dehydrogenase were significantly ($P < 0.05$) increased in infected animals as compared to healthy animals. This study is the first molecular and hematobiochemical evidence of *Babesia bovis* in dairy herds of Punjab province, Pakistan.
***Discussion*:** Bovine babesiosis is one of the important tick-borne diseases (TBD) affecting dairy industry. In bovines, among 3 Babesia species that cause the disease *B. bovis* is more pathogenic with high mortality and morbidity. Pakistan is situated in tropical and sub-tropical region where the humidity is high in some part of countries. This high humidity mostly favors the reproduction of the ticks thus higher prevalence of TBDs in this region. Initially the babesiosis was diagnosed by light microscopy using thin blood smear stained with Giemsa stain. Many studies verified that PCR is a more specific and sensitive tool than conventional techniques for the detection of carrier / asymptomatic ruminants. The haemato-biochemical profile is another valuable footprint to track the disease. Keeping in view the above-mentioned fact the present project has been planned to evaluate the haemato-biochemical alteration between health and *Babesia* infected cattle along with the molecular detection of *Babesia* species involved in bovine babesiosis. The mean values of haemato-biochemical parameters in clinically ill and healthy animals revealed that the mean values of hematological parameters like RBCs, Hb, PCV, and HCT were significantly decreased in diseased animals as compared to the healthy ones. All these might be due the fact that the parasite is intra-erythrocytic in nature and destruction of red blood cells results in significant ($P < 0.05$) decrease level of all the hematological parameters. The mean value of ALT in babesiosis infected cattle was significantly higher as compared to healthy cattle. The mean values of AST and LDH in babesiosis infected cows was significantly higher as compared to that in healthy cows. The elevation in liver enzymes in babesiosis may be due to the hepatic damage and lesions induced by the parasite during its multiplication in the blood followed by disturbed liver function. These enzymes are present in high concentrations in the muscles and liver. High level of these enzymes in the blood is indicator of organ necrosis or damage.

Keywords: bovines, buffaloes, tick-borne diseases, babesiosis, liver enzymes, PCR.

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INTRODUCTION

Livestock sector is one of the fastest growing sectors in Pakistan and plays a vital role in the economy of a country by improving socioeconomic status of poor farmers [29]. Rural economy is largely dependent upon this sector this can be estimated from the fact that each family in rural areas has 2 to 3 large animals to meet their daily expense by selling the milk and its byproducts [9,29-31]. This sector contributed 11.11% in national GDP with a growth rate of 3.76% as compared to last year along with its 58.92% share in agriculture during 2019-20 (Pakistan economic survey 2019-20). Geographically, Pakistan is situated in the tropical and subtropical regions of the world where the tick infestations is very common problem facing by the dairy industry [13,25].

Clinical signs of babesiosis include intermittent high fever (~ 40°C), hemolytic anemia, force breathing, loss of appetite, hemoglobinuria interruption of rumination weakness and sometimes abortion in pregnant females [1,16,21,24,33]. Exotic breeds of cattle are more susceptible to babesiosis as compared to indigenous breed [4,27]. Many other diagnostic tests like haemato-biochemical, serological, and molecular tests must use for the confirmation in addition to diagnosis [3,12,26]. Other serological tests like ELISA and IFAT are also used for the diagnosis of bovine babesiosis [2,7,29]. Haemato-biochemical investigations are also useful in the diagnosis of bovine babesiosis [17]. The present study is designed to investigate the molecular prevalence of *Babesia* and comparative alteration of haemato-biochemical parameters in cows and buffaloes.

MATERIALS AND METHODS

Sample collection and screening

The current study was carried out in year of 2018/2019 in both seasons on the cattle and buffalo's population in target area. A total of 259 blood samples buffaloes (n = 79) and cows (n = 180) were collected using multistage sampling from 3 districts i.e., Faisalabad (31° 25' 7.3740" N and 73° 4' 44.7924" E), Jhang (31° 16' 40.9656" N and 72° 18' 42.3360" E) and Toba Tek Singh (30° 58' 9.7392" N and 72° 27' 40.7484" E) of central Punjab, Pakistan. The samples were collected from apparently healthy animals from jugular vein. First, the samples were subjected to microscopic analysis for these 2 blood smears were made and fixed in methanol[1] (100%) and Giemsa[2] stained by following all the procedures and protocol mentioned [20]. Additionally, 3 mL of blood collected from the same animals in vacutainer[3] containing EDTA and gel clot for further haemato-biochemical and molecular investigations.

DNA extraction and purity analysis

All the blood samples which showed positive results for *Babesia* under microscope[3] were further subjected to DNA extraction, inorganic method (Raw) of DNA extraction was used to extract the DNA by adopting all the procedures and protocols as described [30] spectrophotometry[4] technique was used to check the quality of extracted DNA in for concentration (ng/µL) and purity by using 260/280 wavelength ration, assessing the extracted DNA is suitability for PCR analysis.

PCR analysis

The isolated DNA from positive samples were further assessed to PCR analysis targeting the Bbo gene by using the primers[5] pair reported by [28] in forward and reverse sequence (Forward Primer; 5'- TGAA-CAAAGCAGGTATCATAGG-3' and the Reverse Primer; 5'- CCAAGGAGATTGTGATAATTCA-3') (Gene bank number/Accession number AF053002). PCR Master mix[4] was used and PCR mixture was prepared in final volume of 25 µL. Reaction was carried out in a thermocycler[6] in 35 cycles after initial denaturation of DNA at 95°C for 5 min with denaturation at 95°C followed by annealing at 60 °C and extension was set at 70°C finally the elongation was set at 72°C for 10 min. Both the positive and negative control was run in every step. For the visual results 1.5% ethidium bromide[1]-stained gel is prepared and molecular marker of 100 bp is used to read the expected results after gel electrophoresis. The band observed at bp were considered as positive for *Babesia*.

Hematological and biochemical investigations

Hematology of all the positive blood samples were carried out by using automatic hematological analyzer[7] (Medonic®). The hematological parameters under consideration during the current study were includes RBCs, HB, PCV, MCH and MCHC.

For the serum biochemistry analysis, the samples collected in gel clot tubes were centrifuged 2000 x *g* for 10 min and serum was separated, the separated serum was stored at -40°C for further analysis. The serum biochemistry analysis was carried out by using automatic serum biochemistry analyzer[8] (Micorlab 300)

by kit method by following all manufacturer protocols. The serum biochemistry parameters were studied during current study includes ALT, AST, Cholesterol and LDH.

RESULTS

In this study a total of 518 blood samples in both seasons (summer + winter) from bovine were collected i.e., buffaloes (n=79), cows (n=180) residing in Faisalabad, Toba Tek Singh and Jhang districts in Punjab, Pakistan. First, the samples were screened via the blood film under microscope in both seasons (summer & winter) and the blood smears examination revealed parasite as tear drop in pair identified as *Babesia* and the overall microscopic prevalence in summer was 13.92% (11/79) and 20.55% (37/180) in buffaloes and cows, respectively and in winter was 5.06% (4/79) and 8.88% (16/180) in buffaloes and cows, respectively. Furthermore, the prevalence based upon different risk factors i.e., age, sex, housing, floor system, presence or absence of ticks was also determined.

The district wise prevalence of babesiosis in buffaloes and cows was also vary in both seasons i.e., in summer in buffaloes, prevalence was 10.71 (3/28), 14.28% (3/21) and 16.66% (5/30) in Faisalabad, Toba Tek Singh and Jhang respectively, in cows the prevalence was 20.00 (12/60), 12.50 (6/48) and 26.38% (19/72) in Faisalabad, Toba Tek Singh and Jhang, respectively. While in winter in buffaloes the prevalence was 3.57 (01/28), 0.00 (0/21) and 10.00% (3/30) in Faisalabad, Toba Tek Singh and Jhang, respectively, while in cows 8.33 (5/60), 6.25 (3/48) and 11.11% (8/72) in Faisalabad, Toba Tek Singh and Jhang respectively (Table 1) [Figure 1]. Molecular results of current study revealed that, in summer 24.05% (19/79) and 29.4% (53/180) of buffaloes and cows' populations were positive through standard PCR while in winter the molecular prevalence was in 13.92 % (11/79) and 12.7% (23/180) in buffaloes and cows, respectively (Figure 2).

The positive samples were further investigated for hematological and biochemical analysis. The results revealed that, the mean value of hematological parameters like RBCs, Hb, PCV, MCV and MCHC was statistically significant ($P < 0.05$) and decreased in infected animals (cows and buffaloes) as compared to the non-infected ones in (Tables 2 & 3) [Figures 3 & 4]. In Figures 3, 4, 5, and 6 the columns bearing all the parameters of the infected one are the statistically significant ($P < 0.05$) to the columns bearing the parameters of non-infected one in both seasons. While the biochemical parameters like ALT, AST, Cholesterol and LDH were significantly ($P < 0.05$) increased in infected animals as compared to healthy animals (Tables 4 & 5) [Figures 5 & 6].

Table 1. Prevalence of babesiosis in large ruminants in summer and winter season based upon microscopy in Faisalabad, Toba Tek Singh and Jhang of Punjab province, Pakistan.

Districts	Variables	Prevalence (%)	Chi- Square	Odds Ratio	95% C.I.		*P*-Value
					Lower	Upper	
Summer							
Faisalabad	Cow Buffaloes	12/60 (20.00) 3/28 (10.71)	13.32	0.78	(0.45	0.97)	0.043
Toba Tek Singh	Cow Buffaloes	6/48 (12.50) 3/21 (14.28)	18.95	0.93	(0.35	1.15)	0.049
Jhang	Cow Buffaloes	19/72 (26.38) 5/30 (16.66)	21.74	1.49	(1.32	1.54)	0.039
Total	Cow Buffaloes	37/180 (20.55) 11/79 (13.92)	20.42	1.78	(1.47	1.90)	0.020
Winter							
Faisalabad	Cow Buffaloes	5/60(8.33) 1/29 (3.57)	23.52	1.59	(1.48,	1.88)	0.040
Toba Tek Singh	Cow Buffaloes	3/48 (6.25) 0/21 (0.00)	19.42	0.84	(0.47,	0.90)	0.038
Jhang	Cow Buffaloes	8/72 (11.11) 3/30 (10.00)	20.03	1.30	(1.14,	1.45	0.049
Total	Cow Buffaloes	16/180 (8.88) 4/79 (5.06)	16.32	0.64	(0.34,	0.80)	0.035

$P \leq 0.05$ = Significant; $P \geq 0.05$ = Non-Significant; C.I. = 95%.

Table 2. Hematological analysis in cows in both seasons (summer and winter) in Punjab province, Pakistan.

Summer (Infected = 37; non-infected = 143)				
Parameter	Category	Mean + SD	t-value	*P*-Value
Red Blood Cells (RBCs) (10^6/µL)	Infected	3.2 ± 0.75	-32.82	0.02
	Non-infected	7.5 ± 0.53		
Hemoglobin (Hb) (g/dL)	Infected	5.36 ± 0.74	-53.23	0.032
	Non-infected	12.00 ± 0.33		
Packed Cell Volume (PCV) (%)	Infected	17.22 ± 0.64	-80.57	0.037
	Non-infected	26.7 ± 0.63		
Mean Corpuscular Hemoglobin (MCH) (g/dL)	Infected	7.40 ± 0.73	-49.89	0.022
	Non-infected	13.5 ± 0.28		
Mean Corpuscular Hemoglobin Concentration (MCHC) (Pg)	Infected	20.0 ± 0.64	-81.64	0.019
	Non-infected	30.0 ± 0.75		
Mean Corpuscular Volume (MCV) (%)	Infected	24.5 ± 0.73	-139.68	0.026
	Non-infected	42.0 ± 0.43		
Winter (Infected = 16; Non-infected = 164)				
Red Blood Cells (RBCs) (10^6/µL)	Infected	3.31 ± 0.23	-52.09	0.030
	Non-infected	7.00 ± 0.53		
Hemoglobin (Hb) (g/dL)	Infected	5.75 ± 0.43	-41.75	0.026
	Non-infected	10.7 ± 0.64		
Packed Cell Volume (PCV) (%)	Infected	16.63 ± 0.74	-46.42	0.040
	Non-infected	25.43 ± 0.53		
Mean Corpuscular Hemoglobin (MCH) (g/dL)	Infected	8.81 ± 0.17	-16.69	0.041
	Non-infected	9.8 ± 0.53		
Mean Corpuscular Hemoglobin Concentration (MCHC) (Pg)	Infected	22.4 ± 0.74	-18.99	0.036
	Non-infected	26.0 ± 0.53		
Mean Corpuscular Volume (MCV) (g/dL)	Infected	29.1 ± 0.36	-144.74	0.029
	Non-infected	44.0 ± 0.64		

Table 3. Hematological analysis in buffaloes in both seasons (summer and winter) in Punjab province, Pakistan.

Summer (Infected = 11; Non-infected = 68)				
Parameter	Category	Mean + SD	t-value	*P*-Value
Red Blood Cells (RBCs) (10^6/µL)	Infected	4.0 ± 0.74	-17.36	0.041
	Non-infected	7.9 ± 0.22		
Hemoglobin (Hb) (g/dL)	Infected	4.9 ± 0.64	-37.10	0.032
	Non-infected	12.6 ± 0.63		
Packed Cell Volume (PCV) (%)	Infected	20.5 ± 0.74	-66.57	0.042
	Non-infected	35.5 ± 0.26		
Mean Corpuscular Hemoglobin (MCH) (g/dL)	Infected	6.02 ± 0.53	-40.61	0.041
	Non-infected	13.3 ± 0.67		
Mean Corpuscular Hemoglobin Concentration (MCHC) (Pg)	Infected	20.6 ± 0.32	-113.08	0.039
	Non-infected	35.5 ± 0.74		
Mean Corpuscular Volume (MCV)(%)	Infected	22.5 ± 0.22	-103.05	0.046
	Non-infected	34.0 ± 0.74		
Winter (Infected = 04; Non-infected = 75)				
Red Blood Cells (RBCs) (10^6/µL)	Infected	3.5 ± 0.44	-23.78	0.046
	Non-infected	8.2 ± 0.16		
Hemoglobin (Hb) (g/dL)	Infected	6.7 ± 0.33	-39.75	0.043
	Non-infected	13.0 ± 0.50		
Packed Cell Volume (PCV) (%)	Infected	17.5 ± 0.44	-87.49	0.047
	Non-infected	35.5 ± 0.52		
Mean Corpuscular Hemoglobin (MCH) (g/dL)	Infected	5.7 ± 0.73	-23.22	0.049
	Non-infected	13.3 ± 0.20		
Mean Corpuscular Hemoglobin Concentration (MCHC) (Pg)	Infected	22.8 ± 0.43	-64.55	0.048
	Non-infected	35.4 ± 0.29		
Mean Corpuscular Volume (MCV) (%)	Infected	21.8 ± 0.27	-79.88	0.048
	Non-infected	32.3 ± 0.45		

Table 4. Biochemical analysis in cows (summer + winter) in Punjab province, Pakistan.

Summer (Infected = 37; non-infected = 143)				
Parameter	Category	Mean + SD	t-value	*P*-Value
Alanine Amino Transferase (ALT) (IU/L)	Infected	56.4 ± 0.65	-10.53	0.016
	Non-infected	38 ± 0.32		
Aspartate Amino Transferase (AST) (IU/L)	Infected	150.8 ± 0.93	-33.53	0.021
	Non-infected	144 ± 0.12		
Cholesterol	Infected	415 ± 0.42	-29.43	0.035
	Non-infected	381.4 ± 0.63		
Lactate Dehydrogenase (LDH)	Infected	1181 ± 0.53	-19.54	0.047
	Non-infected	1032.8 ± 0.42		
Winter (Infected = 16; non-infected = 164)				
Alanine Amino Transferase (ALT) (IU/L)	Infected	48.6 ± 0.43	-32.53	0.034
	Non-infected	34 ± 0.19		
Aspartate Amino Transferase (AST) (IU/L)	Infected	149.8 ± 0.56	-14.64	0.041
	Non-infected	142 ± 0.53		
Cholesterol	Infected	426.3 ± 0.28	-26.43	0.043
	Non-infected	419 ± 0.43		
Lactate Dehydrogenase (LDH)	Infected	1167.2 ± 0.43	-18.53	0.032
	Non-Infected	1025.4 ± 0.43		

Table 5. Biochemical analysis in buffaloes (summer + winter) in Punjab province, Pakistan.

Summer (Infected = 11; Non-infected = 68)				
Parameter	Category	Mean + SD	t-value	*P*-Value
Alanine Amino Transferase (ALT) (IU/L)	Infected	57.5 ± 0.54	-53.22	0.039
	Non-infected	39 ± 0.64		
Aspartate Amino Transferase (AST)(IU/L)	Infected	166.3 ± 0.48	-42.53	0.021
	Non-infected	163 ± 0.02		
Cholesterol mg/dL)	Infected	418.5 ± 0.12	-11.64	0.031
	Non-infected	429 ± 0.63		
Lactate Dehydrogenase (LDH)	Infected	1154.1 ± 0.36	-19.75	0.043
	Non-infected	1134.6 ± 0.69		
Winter (Infected = 04; Non-infected = 75)				
Alanine Amino Transferase (ALT)(IU/L)	Infected	63.3 ± 0.64	-48.64	0.043
	Non-infected	61 ± 0.32		
Aspartate Amino Transferase (AST)(IU/L)	Infected	164.0 ± 0.64	-73.22	0.036
	Non-infected	145 ± 0.75		
Cholesterol (mg/dL)	Infected	430.5 ± 0.46	-22.53	0.039
	Non-infected	431 ± 0.64		
Lactate Dehydrogenase (LDH)	Infected	1149.6 ± 0.54	-29.12	0.049
	Non-infected	1130.6 ± 0.82		

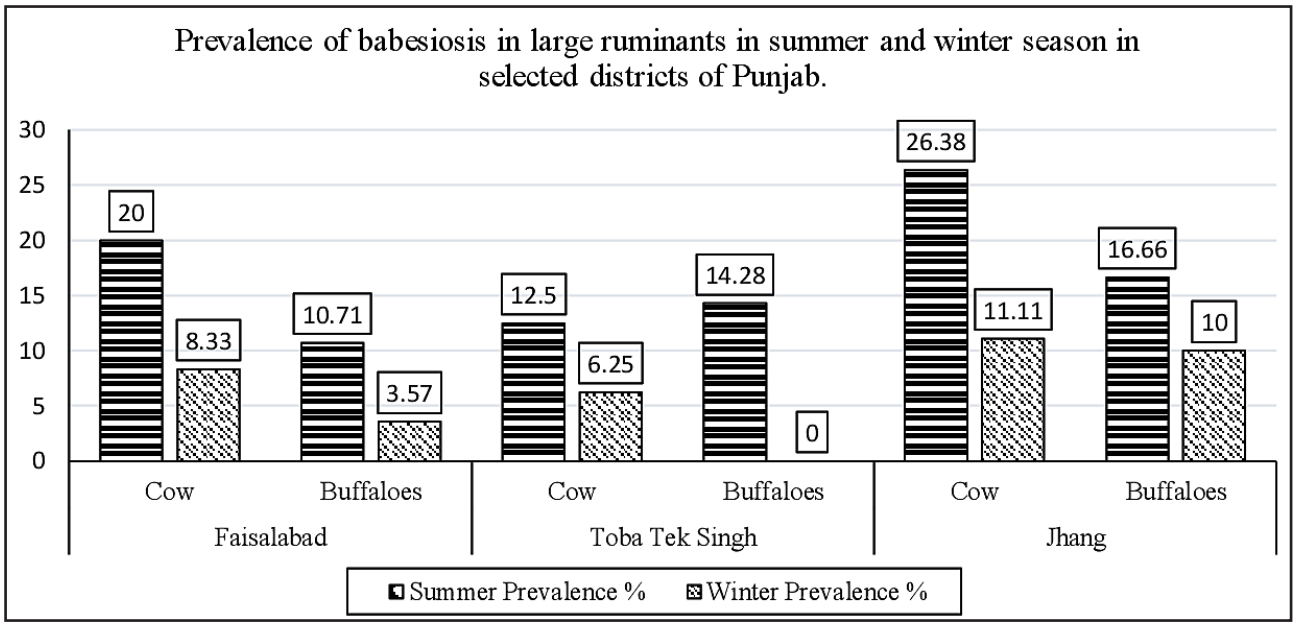
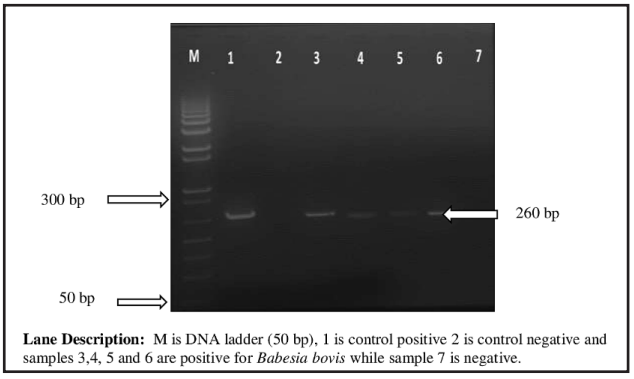


Figure 1. Prevalence of babesiosis in large ruminants in summer and winter season in selected districts of Punjab, Pakistan.



Lane Description: M is DNA ladder (50 bp), 1 is control positive 2 is control negative and samples 3,4, 5 and 6 are positive for *Babesia bovis* while sample 7 is negative.

Figure 2. Photograph of sample showing positive results for *Babesia bovis* (PCR) in large ruminants from selected districts of Punjab, Pakistan.

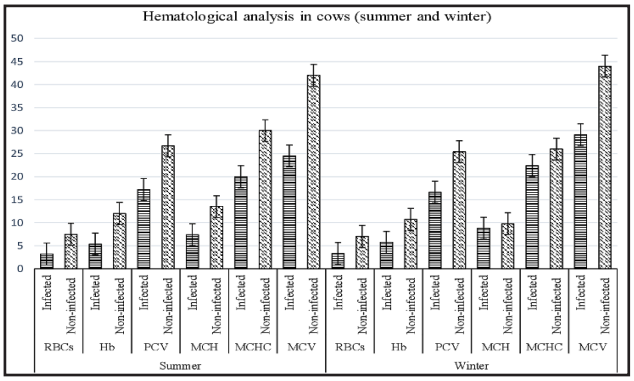


Figure 3. Hematological analysis in cows in both seasons (summer and winter) from selected districts of Punjab, Pakistan.

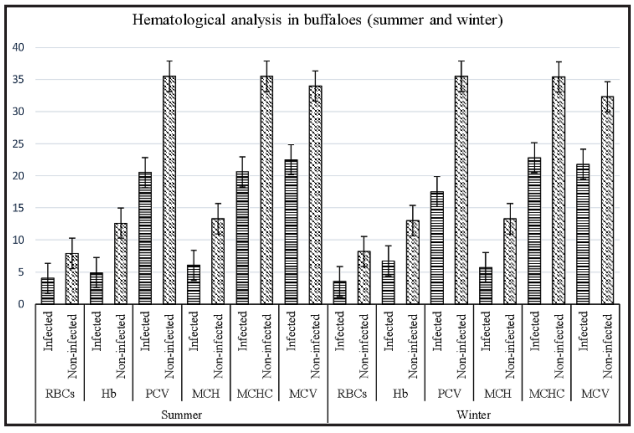


Figure 4. Hematological analysis in buffaloes (summer and winter) from selected districts of Punjab, Pakistan.

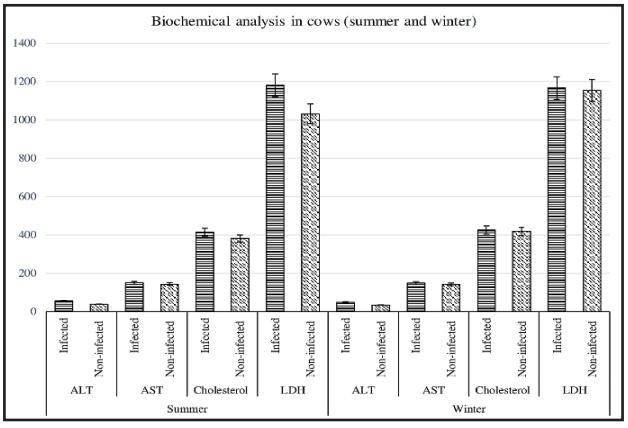


Figure 5. Biochemical analysis in cows (summer + winter) from selected districts of Punjab, Pakistan.

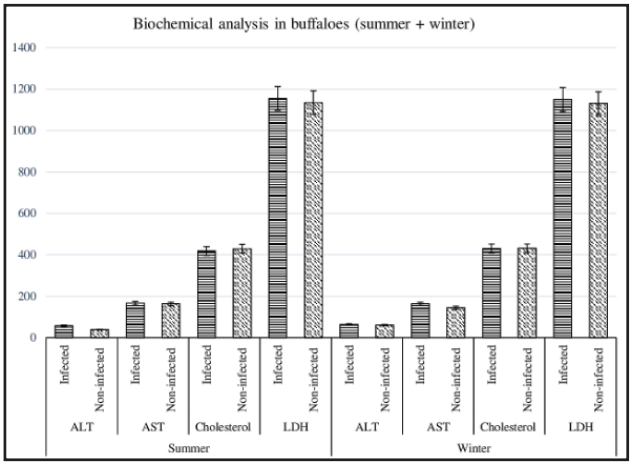


Figure 6. Biochemical analysis in buffaloes (summer + winter) from selected districts of Punjab, Pakistan.

DISCUSSION

Bovine babesiosis is one of the leading tick-borne diseases (TBD) affecting dairy industry. In bovines the main babesia species that cause the disease includes *B. bigemina*, *B. bovis* and *B. divergens* among them *B. bovis* is more pathogenic and cause high mortality and morbidity. The mortality rate ranges between 20-50% in young and sexually mature animals. Exotic dairy cattle are more sensitive to tick infestations as compared to indigenous breeds. *Babesia* is a hemoparasite that is transmitted by the soft tick named ixodid [2]. Pakistan is situated in tropical and sub-tropical region where the humidity is high in some part of countries high humidity is basically favors the growth of such kind of ticks that's why the chances of disease prevalence is higher in this region [18]. The main clinical signs associated with babesiosis in bovines includes high fever, paleness of mucous membrane, weakness animal unable to walk freely and loss of rumination [29].

If we want to decrease the economic losses due to babesiosis, it is need of the hour to develop such a unique diagnostic regimes and assays that not only detects the babesia at very early stage but also provide more accurate results both for the carriers and ongoing parasitic infestations. Initially the babesiosis was diagnosed by using light microscopy for this a drop of blood is needed and from this a thin blood film was formed and stained with Giemsa and examined under the light microscope [7], one major limitation of this technique is this that this is useful only for the acute infestation's cases but not for the carriers' animals, so the threat of being spreading of the disease is still there [15,24,27]. Many studies verified that PCR is a more specific and sensitive tool than conventional techniques for the detection of carrier ruminants having *Babesia* sp. present in blood without any apparent signs of babesiosis [28,32,34].

We had similar experience as the prevalence of *Babesia* detected through PCR was higher as compared with parasitic detection by microscopic examination of Giemsa-stained blood smears. Only microscopic examination of PCR positive samples would have declared many of the positive samples as parasite free. A similar comparison was determined in Kasur, Pakistan [6]. They found 33.3% prevalence for *B. bovis* and *B. bigemina* in cattle from Kasur by PCR as compared with 3% prevalence of *B. bovis* detected by blood smear examination. In the current infection of babesiosis in dairy animals the analysis of haemato-biochemical profile is another valuable footprint to track the disease by keeping in view the above-mentioned fact the present project has been planned to evaluate the haemato-biochemical alteration between health and babesia infected cattle's along with the molecular detection of *Babesia* species involved in bovine babesiosis.

The mean values of haemato-biochemical parameters in clinically ill and healthy animals in our findings revealed that the mean values of hematological parameters like Red blood cells (RBCs), hemoglobin (Hb), Pack cell volume (PCV) and hematocrit (HCT) were significantly decreased in diseased animals as compared to the healthy ones. All these might be due the fact that the parasite is intra-erythrocytic in nature and as a result there is destruction of red blood cells and as a result level of all the hematological parameters are significantly decreased ($P < 0.05$). These results are in agreement with the similar findings already described in the literature [19,23].

The Mean value of Serum Alanine aminotransferase (ALT) in babesiosis infected cattle was significantly higher as compared to that in healthy cattle. The observations recorded in the present study are in agreement with previous results [1,5,14,18,38]. Stated that the elevation in liver enzymes in babesiosis

may be due to the hepatic damage and lesions induced by the parasite during its multiplication in the blood followed by disturbed liver function.

The Mean values of Serum Aspartate aminotransferase (AST) and Lactate dehydrogenase (LDH) in babesiosis infected cows was significantly higher as compared to that in healthy cows. The observations recorded in the present study agree with previous findings [8,10,11,18,32]. Serum AST and ALT concentrations are the indicators of hepatic function and the rise in serum ALT and AST may be due to alteration of liver function as a result of bovine babesiosis [35,36]. These enzymes are present in high concentrations in the muscles and liver. Elevation of these enzymes in the blood is indicator of organ necrosis or damage [22]. *Babesia bigemina* infection causes increase in enzyme activity which may attribute to severe anemia that leads to hypoxic and toxic liver damages. Also, massive hemolysis may occur which in conjunction with hypoxia may lead to hepatic cell degeneration leading to increase in AST, ALT and LDH [28,37].

CONCLUSIONS

In the present study, *Babesia* infection affects the hematobiochemical parameters which is manifested as anemia, low hematocrit value, decrease hemoglobin and PCV. Besides this high level of AST, ALT and LDH were also evaluated. These findings suggest that Babesiosis affects the liver of the affected animals which is shown in the present study. These valuable data obtained in the present study may be helpful in the diagnosis and treatment (symptomatic) of the disease.

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***Declaration of interest*.** The authors declare no conflicts of interest related to this report. The authors alone are responsible for the content and writing of paper.

REFERENCES

1 **AbouLaila M., AbdEl-Aziz A.R., Menshawy S., Yokoyama N., Igarashi I., Al-Wabel M. & Omar M. 2021.** Evaluation of the inhibitory effects of coumermycin A1 on the growth of *Theileria* and *Babesia* parasites *in-vitro* and *in-vivo*. *Pakistan Veterinary Journa*l. 41(4): 469-474.

2 **Ali H., Yousef C.S. & Ali S. 2013.** Assessment of serum antioxidant enzymes activity in cattle suffering from Theileriosis. *European Journal Experimental Biology*. 3(1): 503-507.

3 **Aulakh G.S., Singla L.D., Kaur P. & Alka A. 2005.** Bovine babesiosis due to *Babesia bigemina*: Haematobiochemical and therapeutic studies. *Indian Journal of Animal Science*. 75(6): 617-622.

4 **Babes V. 1888.** Sur hemoglobinuria bacteriome du boeuf [Bacterial hemoglobinuria in cattle]. *Rendus de l'Académie des Sciences III Vie*. 107: 692-694.

5 **Bork S., Okamura M., Boonchit S., Hirata H., Yokoyama N. & Igarashi I. 2004.** Identification of *Babesia bovis* L-lactate dehydrogenase as a potential chemotherapeutical target against bovine babesiosis. *Molecular and Biochemical Parasitology*. 136(2): 165-172.

6 **Ceylan O., Uslu A., Oztürk O. & Sevinc F. 2021.** Serological investigation of some vectorborne parasitic and rickettsial agents in dogs in the western part of Turkey. *Pakistan Veterinary Journal*. 41(3): 386-392.

7 **Durrani A.Z. & Kamal N. 2008.** Identification of ticks and detection of blood protozoa in Friesian cattle by polymerase chain reaction test and estimation of blood parameters in district Kasur, Pakistan. *Tropical Animal Health Production*. 40: 441-447.

8 **Esmaeilnejad B., Tavassoli M. & Asri-Rezaei S. 2012.** Investigation of hematological and biochemical parameters in small ruminants naturally infected with *Babesia ovis*. *Veterinary Research Forum*. 3(1): 31-36.

9 **Farooq R., Hafeez M.A., Oneeb M., Rafique A., Ashraf K., Aslam F., Rauf N., Khalid K., Bilal F., Mahmood M.S. & Sattar A. 2020.** Molecular characterization and phylogenetic analysis of *Babesia* species isolated from domestic cattle. *Pakistan Veterinary Journal*. 40(2): 224-228.

10 **Ganguly A., Bisla R.S., Ganguly I., Singh H., Bhanot V. & Chaudhri S. 2017.** Direct blood PCR detection of *Babesia bigemina* and its effect on hematological and biochemical profile in crossbred cattle of eastern Haryana. *Indian Journal of Animal Research*. 51(1): 141-145.

11 **Ghirbi Y.M., Hurtado A., Brandika J., Khlif K., Ketata Z. & Bouattour A. 2008.** A molecular survey of *Theileria* and *Babesia* parasites in cattle, with a note on the distribution of ticks in Tunisia. *Parasitology Research*.103: 435-442.

12 **Gungi S., Haritha G.S. & Kumari K.N. 2016.** Clinical management of Babesiosis in cattle: A case report. *Research Journal for Veterinary Practitioners*. 4(2): 30-33.

13 **Hussein A.H., Mohammed N.A.E.S. & Mohammed H.K. 2007.** Theileriosis and babesiosis in cattle: haemogram and some biochemical parameters. In: *Proceedings of XIII International Congress of International Society of Animal Hygiene*. 143-150.

14 **Ibrahim O., Taha Z. & Jassim S. 2012.** Prevalence of *Babesia bovis* in cattle in Tikreet city and its surroundings with hematological study. *Tikreet Journal of Pure Sciences*. 17(2): 32-34.

15 **Iqbal F., Fatima M., Shahnawaz S., Naeem M., Shaikh R.S. & Shaikh A.S. 2011.** A study on the determination of risk factors associated with babesiosis and prevalence of *Babesia* sp., by PCR amplification, in small ruminants from southern Punjab (Pakistan). *Parasite*. 18: 229-234.

16 **Jayalakshmi K., Sasikala M., Kavitha S., Ravi R., Veeraselvam M. & Krishnakumar S. 2017.** Hematological and Biochemical Alterations in Babesiosis of Crossbred Cow. *Indian Veterinary Journal*. 94(4): 81-82.

17 **Kerr M.G. 2007.** *Laboratory Medicine Clinical Biochemistry and Hematology*. 2nd edn. Cornwall: Blackwell Science, pp.74-79.

18 **Khan A., Noushin R., Attaullah M., Khan S.N., Hussain R., Dawar F.U., Rehman F.U., Ijaz M. & Ullah K. 2020.** Prevalence of tick born *Babesia* infection in domestic cattle of Khyber Pakhtunkhwa, Pakistan. *Pakistan Journal of Zoology*. 52(6): 2401-2403.

19 **Li K., Zhang L., Luo H., Mehmood K., Shahzad M. & Naseer M.U. 2019.** Besnoitiosis: an emerging parasitic disease in yaks (*Bos grunniens*) and tibetan sheep (*Ovis aries*) on the qinghai tibetan plateau, China. *Pakistan Veterinary Journal*. 39(3): 447-450.

20 **Lotfollahzadeh S., Rahmani M., Mohri M. & Madadgar O. 2012.** Changes in serum iron concentration and hepatic enzyme activities in cattle infected with *Theileria annulata* and *Babesia bigemina*. *Common Clinical Pathology*. 21(5): 829-832.

21 **Mahmoud M.S., Kandil O.M., Nasr S.M., Hendawy S.H., Habeeb S.M. & Mabrouk D.M. 2015.** Serological and molecular diagnostic surveys combined with examining hematological profiles suggests increased levels of infection and hematological response of cattle to babesiosis infections compared to native buffaloes in Egypt. *Parasites & Vectors*. 8(1): 319.

22 **Mohsin M., Li L., Huang X., Aleem M.T., Habib Y.J., Shehata A.I., Afzal M.Z., Abbas R.Z., Abbas A. & Yin G. 2021.** Immunogenicity and protective efficacy of probiotics with EtIMP1C against Eimeria tenella challenge. *Pakistan Veterinary Journal*. 41(2): 274-278.

23 **Moryani A.A., Rajput N., Naeem M., Shah A.H. & Jahejo A.R. 2021.** Screening of the herbs and evaluation of their combined effects on the health and immunity of coccidiosis challenged broiler chickens. *Pakistan Veterinary Journal*. 41(2): 228-234.

24 **Murray R.K., Granner D.K., Mayes P.A. & Rodwell V.W. 1990.** *Harper's Biochemistry*. 26th edn. New York: McGraw-Hill Companies, pp.218-221.

25 **Radostits O.M., Gay C.C., Constable P.D. & Hinchcliff K.W. 2007.** *Veterinary Medicine: A textbook of the disease of cattle, sheep, pigs, goats and horses*. 9th edn. Philadelphia: WB Saunders Elsevier Ltd., pp.29-35.

26 **Razavi S.M., Nazifi S., Rakhshandehroo E., Firoozi P. & Farsandaj M. 2012.** Erythrocyte antioxidant systems, lipid peroxidation and circulating lipid profiles in cattle naturally infected with *Theileria annulata*. *Revue de Médecine Vétérinaire*. 163: 18-24.

27 **Rehman W.U., Khan I.A., Qureshi A.H. & Hussain S. 2004.** Prevalence of different species of Ixodidae (hard ticks) in Rawalpindi and Islamabad. *Pakistan Journal of Medical Sciences*. 43: 42-46.

28 **Romero-Salas D., Mira A., Mosqueda J., Garcia V.Z., Hidalgo R.M., Vela N.A.O., de Leon A.A.P., Florin C.M. & Schnittger L. 2016.** Moleuclar and serological detection of *Babesia bovis* and *Babesia bigemina* infection in bovines and water buffaloes raised jointly in an endemic field. *Veterinary Parasitology* 217: 101-107.

29 Saud N., Ahmed F.A., Sheikh I.U. & Bhattacharya M. 2005. Prevalence of bovine babesiosis in Dirang valley of Arunachal Pradesh. *Indian Veterinary Journal*. 82: 1011-1012.

30 Shahnawaz S., Ali M., Aslam M.A., Fatima R., Chaudhry Z.I. & Hassan M.U. 2011. A study on the prevalence of a tick transmitted pathogen, *Theileria annulata*, and hematological profile in cattle from Southern Punjab (Pakistan). *Parasitology Research*. 109(4): 1155-1160.

31 Shahzad W., Haider N., Mansur-ud-Din A., Munir R., Saghar M.S., Mushtaq M.H. & Mehmood F. 2013. Prevalence and molecular diagnosis of *Babesia ovis* and *Theileria ovis* in Lohi sheep at livestock experiment station (LES), Bahadurnagar, Okara, Pakistan. *Iranian Journal of Parasitology*. 8: 570-587.

32 Shaikh R., Ramzan K., Nazil S., Sattar S., Khan S.N. & Raizuddin S. 2005. A new locus for non-syndromic deafness DFNB51 maps to chromosomes. *American Journal of Medical Genetics A* .138: 295-392.

33 Sharma A., Singla L.D., Ashuma Batth B.K. & Kaur P. 2016. Clinicopathobiochemical alterations associated with subclinical babesiosis in dairy animals. *Journal of Arthropod Borne Diseases*. 10: 259-267.

34 Swar S.O., Abbas R.Z., Asrar R., Yousuf S., Mehmood A., Shehzad B., Farhan H.R., Aleem M.T., Marcelino L.A & Mohsin M. 2021. Milk adulteration and emerging health issues in humans and animals (a review). *Continental Veterinary Journal*. 1(1): 1-8.

35 Talkhan O.F.A., Radwan M.E.I. & Ali M.A. 2010. Cattle babesiosis and associated biochemical alteration in Kalubyia Governorate. *Natural Sciences*. 12: 24-27.

36 Tufani N.A., Fazili M.R., Malik H.U., Beigh S.A. & Dar K.H. 2015. Clinico hematological Profile and therapeutic management of acute babesiosis in a Holstein-Friesian crossbred cow. *Veterinary Clinical* Sciences 3(3): 11-14.

37 Ziapour S.P., Esfandiari B. & Youssefi M.R. 2011. Study of the prevalence of babesiosis in domesticated animals with suspected signs in Mazandaran province, north of Iran, during 2008. J*ournal of Animal and Veterinary Advances.* 10: 712-714.

38 Zulfiqar S., Shahnawaz S., Ali M., Bhutta A.M., Iqbal S., Hayat S. & Iqbal F. 2013. Detection of *Babesia bovis* in blood samples and its effect on the hematological and serum biochemical profile in large ruminants from Southern Punjab. *Asian Pacific Journal of Tropical Biomedicine*. 2: 104-108.