ISSN (Online) = 2522-6754 ISSN (Print) = 2522-6746
WORLD JOURNAL OF BIOLOGY AND BIOTECHNOLOGY
Research Manuscript www.sciplatform.com

Molecular detection of *Babesia bovis* along with haemato-biochemical alterations in large ruminants associated with babesiosis in central Punjab, Pakistan

[a] Mubarik Ali, [b] Muhammad Jamil *, [c] Norina Jabeen, [d] Jaweria Gul, [e] Muhammad Kashif, [f] Imtiaz Khan, [g] Naimat Ullah

[a] Animal Science Institute, National Agricultural Research Center, Islamabad-54000-Pakistan,
[b] PARC Arid Zone Research Center, Dera Ismail Khan, 29050-Pakistan,
[c] Rural Sociology Department, Institute of Social Sciences, University of Agriculture Faisalabad, Pakistan,
[d] Department of Biotechnology, Shaheed Benazir Bhutto University, Sheringal, Dir, Pakistan,
[e] Department of Clinical Sciences, Sub Campus Jhang, University of Veterinary and Animal Sciences, Lahore-54000-Pakistan,
[f] PARC Adaptive Research cum Demonstration Institute Miranshah-28200-Pakistan,
[g] Department of Parasitology, University of Veterinary and Animal Sciences Lahore-54000-Pakistan.

Authors' Contribution | **Ali, M,, M. Jamil, N. Jabeen & N.Ullah** conducted the research; **J. Gul & M. Kashif** procced the data analysis and **I. Khan** wrote the manuscript.

*Corresponding Author's Email Address | jamilmatrah@gmail.com | **Review Proccess: peer review**

Digital Object Identifier (DOI) Number: **https://dx.doi.org/10.33865/wjb.007.02.0587**

ABSTRACT

Babesiosis is endemic in Pakistan and is one of the most important bovine diseases that cause huge economic losses and high mortality in young animals. A hematobiochemical study was conducted to unveil the difference between diseased and healthy animals in specific districts i.e., Faisalabad, Toba Tek Singh and Jhang of Punjab, Pakistan. The total number of blood samples collected were 518 which include samples of 158 buffaloes and 360 cattle. The collected samples are then analyzed with the help of PCR (Polymerase Chain Reaction) targeting apocytochrome b-genes (*CYTb*) followed by hematobiochemical analysis. The analysis of data was done by the Chi-square test. The PCR tests conducted in summer suggests that 53 out of 180 (29.4%) cows and 19 out of 79 (24.05%) buffaloes are prone to babesiosis. On the other hand, in winter results showed that 12.7 (23/180), 13.92 % (11/79) samples positive for Babesia genus through c-PCR. 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 ALT, AST, Cholesterol and LDH were significantly ($P< 0.05$) increased in infected animals as compared to healthy animals. These findings are the novel molecular and hematobiochemical evidence of *B. bovis* in dairy herds of Punjab province, Pakistan.

Keywords: Babesiosis, PCR, hematobiochemical, *B. bovis*, animals, infected.

INTRODUCTION: Livestock sector is considered to be one of the most important and fast developing sector in Pakistan and plays an important role in the economy of a country by improving socioeconomic status of poor farmers and also plays a key role in assuaging poverty (Shahzad *et al.*, 2013). 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 (Shahzad *et al.*, 2013; Sharma *et al.*, 2016). In the fiscal year of 2019/2020 this sector contributed 14% in national GDP with a growth rate of 2.89% as compared to last year along with its 61.9% share in agriculture. Water buffaloes and cows with an anticipated head of 53.4 and 43.7 million respectively, are main milch animals with annual production of 24.238 to 39.503 million tons of milk (GOP, 2021-22). Depending upon the needs and milk supply according to the needs of public the dairy farms systems are divided in to four categories i.e., smallholder subsistence these systems fulfilled daily household needs only, smallholder market-oriented this system basically fulfilled the needs of family and some extra milk is sold to nearby local milk market: rural commercial dairy farms and systems have large no. of milch animals as compared to the rest of systems and they have surplus amount of milk to fulfill the needs at larger scale and also they have direct link to the well develop milk processing plants; last type of milk systems is *peri urban* they have few no of animals and they keep the animals near to the big cities and supply the milk to urban areas (M'ghirbi *et al.*, 2008; Sharma *et al.*, 2016). Despite of tremendous growth and development in the dairy industry, it is facing some problems as well in the form of tick born diseases, among them tick transmitted blood protozoan is major constrains in the development of this sector (Rehman *et al.*, 2004). 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 (Iqbal *et al.*, 2011).

Bovine babesiosis is one of the leading ticks borne diseases in dairy industry, babesiosis was first identified by Victor Babes in 1888 in Romania (Babes, 1888) and it was the major cause of sudden death of dairy cattle at that time and till date more than 100 species of babesia have identified affecting different livestock species including wild and pet animals (Tufani *et al.*, 2015). Here we engrossed on bovine babesiosis. In cattle babesiosis is mainly caused by the *B. bigemina* and *B. bovis* babesia is an intraerythrocytic parasite and it is transmitted by the bite of soft tick named *Ixodid* demonstrated that it is an arthropod vector transmitted disease (Aziz *et al.*, 2020). Follow up studies were also revealed babesia as tick-transmitted apicomplexan protozoan haemoparasites which have great economic impact worldwide. The main clinical signs in bovines

babesiosis are intermittent high fever (~104 °F), hemolytic anemia, force breathing, loss of appetite, hemoglobinuria interruption of rumination weakness and sometimes abortion occurs in pregnant females due to prolong onset of high fever and animal unable to move (Mosqueda *et al.*, 2012; Razavi *et al.*, 2012). Exotic breeds of cattle are more susceptible to babesiosis as compared to indigenous breed due to this fact high mortality (35-50%) is observed in imported animals (Saud *et al.*, 2005).

Many factors like clinical signs (high fever), season, infestations of ticks are potent indicators for occurrence of disease but other diagnostic tests like haemato-biochemical, serological, and molecular tests must use for the confirmation in addition to diagnosis these tests provide the epidemiological status of disease in a particular area to know the enzootic stability (Ibrahim, 2017). Traditionally bovine babesiosis is diagnosed directly by blood film examinations under microscope and due to quick and inexpensive this microscopic examination is still used (Salem *et al.*, 2016). During acute phase of disease. During acute phase of disease number of parasites are increased to certain a level where they can be easily detected by microscopy drawback of this technique is that it is only useful in active infection not for the subclinical infection (Aulakh *et al.*, 2005). Other serological tests like ELISA and IFAT are also used for the diagnosis of bovine babesiosis these two tests mostly detect antibodies (Immune response) in the serum some time they give the false result due to cross reactivity in addition to this there is short window of infection (12-15 days) (Esmaeilnejad *et al.*, 2012). Molecular tests i.e., standard PCR, nested PCR, LAMP assay and qRT-PCR useful and detects the presence of parasite DNA, which is more confirmatory, but the drawback of these molecular assay are that due to use specific treatment parasite DNA removed from the blood (Hassanpour *et al.*, 2013; Shahzad *et al.*, 2013). In Pakistan all the methods from traditionally blood film examination to molecular methods have been used separately for the diagnosis of bovine babesiosis but not collectively. Hematological and biochemical investigations are also useful in the diagnosis of bovine babesiosis as the parasite is intraerythrocytic in nature.

OBJECTIVES: Very few data regarding the clinicopathology-biochemical and molecular detection investigations of bovine babesiosis is available in Pakistan that's why the current research was envisaged with core objectives of babesias detection in the blood of cows and buffaloes along with the haemato-biochemical investigations of bovine babesiosis.

MATERIALS AND METHODS: Sample collection and initial examination: The present investigation on the number of cattle and buffalo in the target region was conducted in 2018/2019 during both seasons. The blood samples of 259 buffaloes and cows were collected from different districts of Pakistan. The districts from where the samples are collected include Faisalabad, Jhang, and Toba Tek Singh. The samples were collected from apparently healthy animals from jugular vein. First, the samples were subjected to microscopic analysis for these two blood smears were made and fixed in Methanol (100%) and Giemsa stained by following all the procedures and protocol mentioned by Moretti *et al.* (2010). Additionally, 3 mL of blood collected from the same animals in vacutainer 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 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 by Shaikh *et al.* (2005). Spectrophotometry 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 suitability of the extracted DNA 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 pair reported by Salas et al in forward and reverse sequence (Forward Primer; 5'- TGA ACA AAG CAG GTA TCA TAG G-3' and the Reverse Primer; 5'- CCA AGG AGA TTG TGA TAA TTC A-3'). PCR Master mix was used from (Thermoscientific®, USA) and PCR mixture was prepared in final volume of 25 µL. Reaction was carried out in a thermocycle 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 for 1 min. finally the elongation was set at 72 °C for 10 min. Both the positive and negative control was inculded in every step. For the visual results 1.5% ethidium bromide-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 (Medonic®, Sweden). 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 @ 5000 RPM for 10 minutes 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 (MERCK, 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 AND DISCUSSION: This study consists of sample collection in both seasons (summer + winter) from bovine *i.e.*, buffaloes (n=79), cows (n=180) from different Districts of Punjab, Pakistan i.e., Faisalabad, Toba Tek Singh and Jhang. The screening of blood samples via the blood film were the first step in this study 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. *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* (Shahzad *et al.*, 2013).

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 (Lotfollahzadeh *et al.*, 2012). 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 (Shahzad *et al.*, 2013). If we want to decrease the economic losses due to babesiosis, it is need of 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. Additionally, the occurrence according to several risk factors, such as age, sex, housing, floor system, and availability of tick, was also examined. The occurrence of babesiosis was seen to be different in selected districts of Pakistan. 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, 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 Saud *et al.* (2005); (Kerr, 2008; Razavi *et al.*, 2012). The conventional techniques for the detection of carrier ruminants had disadvantage over PCR that the conventional techniques are non-specific and less sensitive (Talkhan *et al.*, 2010; Ziapour *et al.*, 2011). It was also noticed that the season also has an impact on the prevalence of disease. In summer, the prevalence of disease in buffaloes were high in Jhang (16.66%) followed by Toba Tek Singh (14.28%) and Faisalabad (10.71%). In Jhang, 5 out of 30, in Toba Tek Singh 3 out of 21 and in Faisalabad 3 out of 28 buffalo have babesiosis. Similarly, the prevalence of disease in cows in summer were also highest in Jhang (26.38) followed by Faisalabad (20%) and then in Toba Tek Singh (12.50%). In Jhang, 19 out of 72, in Toba Tek Singh 6 out of 48 and in Faisalabad 12 out of 60 buffalo have babesiosis. The results of PCR in winter were different as compared to summer. In winter, the prevalence of disease in buffaloes were high in Jhang (10%) followed by Faisalabad (3.57%) while Toba Tek Singh had zero prevalence. In Jhang, 3 out of 30 and in Faisalabad 1 out of 28 buffalo have babesiosis. Similarly, the prevalence of disease in cows in winter were also highest in Jhang (11.11%) followed by Faisalabad (8.33%) and then in Toba Tek Singh (6.25%). In Jhang, 8 out of 72, in Faisalabad 5 out of 60 and in Toba Tek Singh 13out of 48 buffalo have babesiosis (table 1 & figure 1).

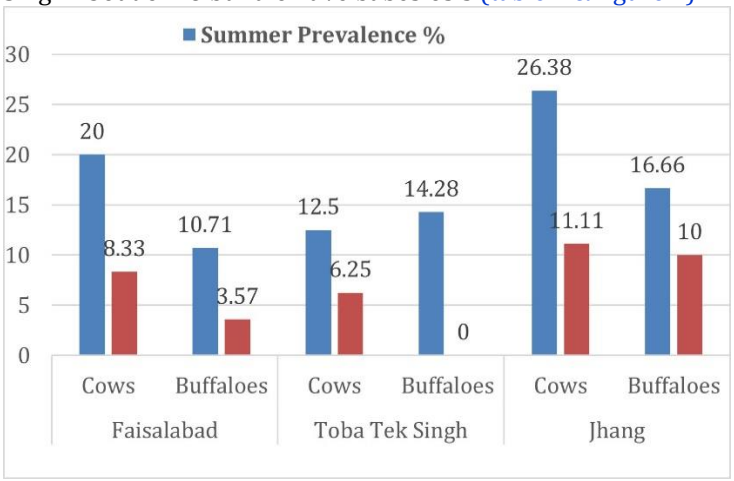

Figure 1: District wise prevalence of babesiosis in large ruminants in summer and winter season.

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

Table 1: District wise prevalence of babesiosis in large ruminants in summer and winter season based upon microscopy.

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 significantly ($P< 0.05$) decreased in infected animals (cows and buffaloes) as compared to the non-infected ones (table 2&3 and figure 3&4). While the biochemical parameters like ALT, AST, Cholesterol

Summer (Infected = 37; non-infected = 143)					
Parameter	**Unit**	**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 2: Hematological Analysis in cows in both seasons (summer and winter).

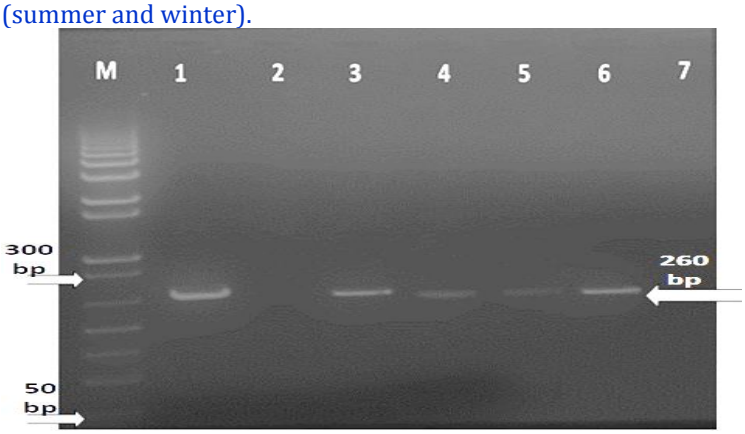

Figure 2: Molecular identification of *Babesia bovis* through PCR assay. 1 is control positive 2 is control negative and samples 3,4, 5 and 6 are positive for Babesia bovis while sample 7 is negative.

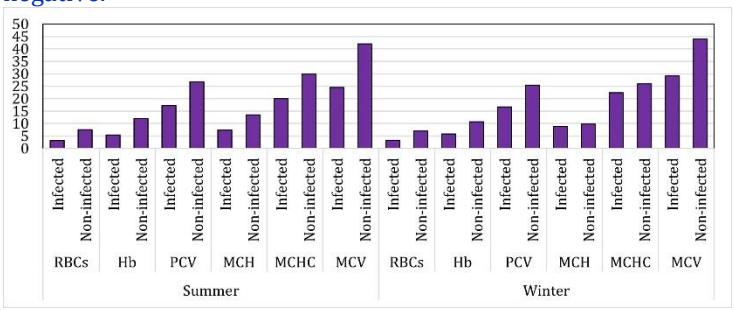

Figure 3: Hematological Analysis in cows in both seasons (summer and winter).

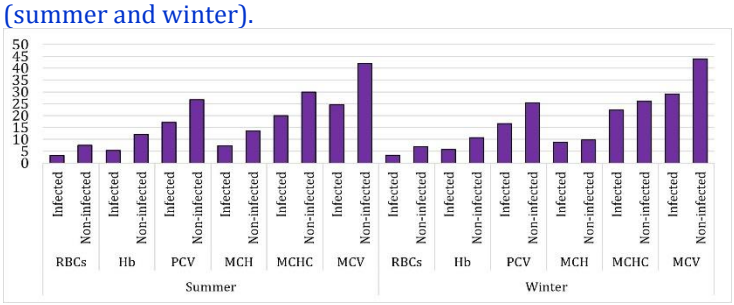

Figure 4: Hematological Analysis in buffaloes (Summer and Winter).

and LDH were significantly (P< 0.05) increased in infected animals as compared to healthy animals (table 4&5 and figure 5&6).

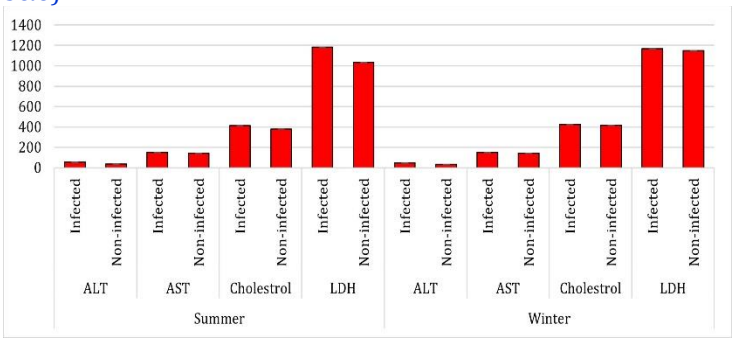

Figure 5: Biochemical analysis in cows (Summer + Winter).

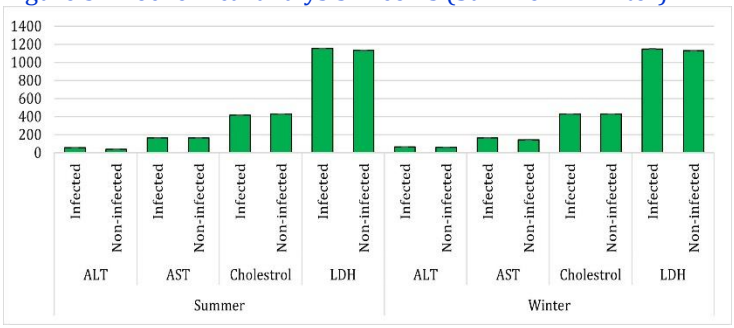

Figure 6: Biochemical analysis in Buffaloes (Summer + Winter).

The prevalence of Babesia detected by microscopic examination of Giemsa-stained blood smears was lower as compared to the detection through PCR. Only microscopic examination of PCR positive samples would have declared many of the positive samples as parasite free. A similar comparison was made by Durrani and Kamal in Kasur, Pakistan (Durrani and Kamal, 2008). They found 33.3% prevalence for *B. bovis* and *B. bigemina* in cattle from Kasure by PCR as compared with 3% prevalence of *B. bovis* detected by blood smear examination. The progression of disease caused by the babesiosis can also be

Summer (Infected = 37; non-infected = 143)					
Parameter	**Unit**	**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).

Summer (Infected = 37; non-infected = 143)					
Parameter	**Unit**	**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)	(IU/L)	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)	(IU/L)	Infected	1167.2±0.43	-18.53	0.032
(IU/L)		Infected	1148.6±0.43	-32.53	0.034
Alanine Amino Transferase (ALT)					

Table 4: Biochemical analysis in cows (Summer + Winter).

tracked by the analyzing the haemato-biochemical profile of affected individual. The mean values of haemato-biochemical parameters in clinically ill and healthy animals are presented in the table, and 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 previous studies (Radostits *et al.*, 2006); Mahmoud *et al.* (2015). Serum Alanine aminotransferase (ALT) is the important indicator for the detection of babesiosis and it was observed that healthy cattle had low level of ALT as compared to the cattle having babesiosis. These observations are consistent with the findings cited in literature Alam and Nasr, (2011); Lotfollahzadeh *et al.*, 2012 and Jayalakshmi *et al.*, 2017). The reason for the increase in ALT in affected individuals may be due the fact that the

Parameter	Unit	Category	Mean + SD	t-value	P-Value
Summer (Infected = 11; non-infected = 68)					
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)	(IU/L)	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)	(IU/L)	Infected	1149.6±0.54	-29.12	0.049
		Non-infected	1130.6±0.82		

Table 5: Biochemical analysis in Buffaloes (Summer + Winter).

parasites damage the hepatic cells and induce lesions which may lead to abnormal ALT. The healthy cows had low mean values of LDH (Lactate dehydrogenase), and AST (Aspartate aminotransferase) as compared to that of cows infected with babesiosis. These observations are consistent with the findings of various researchers Hussein *et al.*, (2007); Gungi *et al.*, (2016) and Ganguly *et al.*, (2017). The hepatic function can be analyzed with the help of serum concentration of ALT and AST. In case of bovine babesiosis, the concentration of ALT and AST rises significantly in the serum (Zulfiqar *et al.*, 2012). The most abundant site for the presence of these enzymes are liver and muscle cells. Necrosis can also be identified by the detection of these enzymes in the serum (Murray *et al.*, 2000). Increased enzymatic reaction brought on by a babesia bigeminal infection may contribute to severe anemia, which causes toxic and hypoxic liver damage. Additionally, significant hemolysis may take place, which may combine with hypoxia to cause hepatic cell degeneration and a rise in AST, ALT, and LDH (Shahnawaz *et al.*, 2011).

CONCLUSION: Babesia infection in the present investigation impacts the hematobiochemical parameters, which expresses as anemia, a low hematocrit value, a drop in hemoglobin, and a decrease in PCV. In addition to the above parameters, LDH, AST, and ALT were observed. Babesiosis majorly affects the liver of an animal which is also noticed in this study. This research will open new paradigm for the symptomatic treatment and diagnosis of diseases.

CONFLICT OF INTEREST: Authors have no conflict of interest.

REFERENCES: Alam, T. and S. Nasr, 2011. Hematological and biochemical investigation in bovine babesiosis and theileriosis. Benha Veterinary Medical journal, 22(2): 118-126.

Aulakh, G., L. Singla, P. Kaur and A. Alka, 2005. *Bovine babesiosis* due to *Babesia bigemina* haematobiochemical and therapeutic studies. The Indian journal of animal sciences, 75(6); 11-25.

Aziz, P., S. Marodia, P. Ganesan and C. Sharma, 2020. A clinical study on hemato-biochemical changes in cows affected with babesiosis. Pharma innovation journal, 9(2): 242-245.

Babes, V., 1888. Sur l'hemoglobinurie bacterienne du boeuf. CR Acad. Sci, 107: 692-694.

Durrani, A. and N. Kamal, 2008. Identification of ticks and detection of blood protozoa in friesian cattle by polmerase chain reacton test and estimation of blood parameters in district kasur, pakistan. Tropical animal health and production, 40(6): 441-447.

Esmaeilnejad, B., M. Tavassoli and S. Asri-Rezaei, 2012. Investigation of hematological and biochemical parameters in small ruminants naturally infected with *Babesia ovis*. In: Veterinary Research Forum. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran: pp: 31.

Ganguly, A., R. Bisla, I. Ganguly, H. Singh, V. Bhanot and S. Chaudhri, 2017. Direct blood PCR detection of *Babesia bigeminaand* its effect on haematological and biochemical profile in crossbred cattle of eastern haryana. Indian journal of animal research, 51(1): 141-145.

Gungi, S., G. Haritha and K. Kumari, 2016. Clinical management of babesiosis in cattle: A case report. Research journal of vetanary practical, 4(2): 30-33.

Hassanpour, A., Y. G. Sabegh, A. Sadeghi-nasab and A. Hassanpour, 2013. Assessment of serum antioxidant enzymes activity in cattle suffering from theileriosis. Europian journal of expertmental biology, 3(1): 493-496.

Hussein, A., N. Mohammed and H. Mohammed, 2007. Theileriosis and babesiosis in cattle: Haemogram and some biochemical parameters. In: Proceedings of XIII International Congress of International Society of Animal Hygiene. pp: 21.

Ibrahim, S. O. A., 2017. *Gastrointestinal helminth* parasites of ruminants slaughtered in Shendi Abattoir, River Nile State, Sudan. Yasser Abdelmaged Suleiman.

Iqbal, F., M. Fatima, S. Shahnawaz, M. Naeem, R. Shaikh, M. Ali, A. Shaikh and M. Aktas, 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: journal de la société française de parasitologie, 18(3): 229.

Jayalakshmi, K., M. Sasikala, S. Kavitha, R. Ravi, M. Veeraselvam

and S. Krishnakumar, 2017. Haematological and biochemical alterations in *Babesiosis* of crossbred cow. Indian veterinary journal, 94(04): 81-82.

Kerr, M. G., 2008. Veterinary laboratory medicine: Clinical biochemistry and haematology. John Wiley & Sons.

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

M'ghirbi, Y., A. Hurtado, J. Brandika, K. Khlif, Z. Ketata and A. Bouattour, 2008. A molecular survey of *Theileria* and *Babesia* parasites in cattle, with a note on the distribution of ticks in Tunisia. Parasitology research, 103(2): 435-442.

Mahmoud, M. S., O. M. Kandil, S. M. Nasr, S. H. Hendawy, S. M. Habeeb, D. M. Mabrouk, M. G. Silva and C. E. Suarez, 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): 1-15.

Moretti, A., V. Mangili, R. Salvatori, C. Maresca, E. Scoccia, A. Torina, I. Moretta, S. Gabrielli, M. P. Tampieri and M. Pietrobelli, 2010. Prevalence and diagnosis of *Babesia* and *Theileria* infections in horses in italy: A preliminary study. The veterinary journal, 184(3): 346-350.

Mosqueda, J., A. Olvera-Ramirez, G. Aguilar-Tipacamu and G. J Canto, 2012. Current advances in detection and treatment of babesiosis. Current medicinal chemistry, 19(10): 1504-1518.

Murray, R. K., D. K. Granner, P. Mayes and V. W. Rodwell, 2000. Harper's biochemistry, appleton and lange. Stamford, CT.[Google Scholar].

Radostits, O. M., C. Gay, K. W. Hinchcliff and P. D. Constable, 2006. Veterinary medicine e-book: A textbook of the diseases of cattle, horses, sheep, pigs and goats. Elsevier health sciences.

Razavi, S., S. Nazifi, E. Rakhshandehroo, P. Firoozi and M. Farsandaj, 2012. Erythrocyte antioxidant systems, lipid peroxidation and circulating lipid profiles in cattle naturally infected with theileria annulata. Review medical vetarnary, 163(1): 18-24.

Rehman, W., I. A. Khan, A. Qureshi and S. Hussain, 2004. Prevalence of different species of ixodidae (hard ticks) in Rawalpindi and Islamabad. Pakistan journal of medical science, 43: 42-46.

Salem, N. Y., S. G. Yehia, H. S. Farag and M. A. Elkhiat, 2016. Clinical, hemato-biochemical alterations and oxidant–antioxidant biomarkers in babesia-infected calves. International journal of veterinary science and medicine, 4(1): 17-22.

Saud, N., F. Ahmed, I. Sheikh and M. Bhattacharya, 2005. Prevalence of bovine babesiosis in dirang valley of arunachal pradesh. Indian veterinary journal, 82(9): 1011-1012.

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

Shahzad, W., N. Haider, A. Mansur-ud-Din, R. Munir, M. S. Saghar, M. H. Mushtaq, N. Ahmad, G. Akbar and F. Mehmood, 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(4): 570.

Shaikh, R. S., K. Ramzan, S. Nazli, S. Sattar, S. N. Khan, S. Riazuddin, Z. M. Ahmed, T. B. Friedman and S. Riazuddin, 2005. A new locus for nonsyndromic deafness dfnb51 maps to chromosome 11p13-p12. American journal of medical genetics. Part A, 138(4): 392.

Sharma, A., L. D. Singla, B. K. Batth and P. Kaur, 2016. Clinicopatho-biochemical alterations associated with subclinical babesiosis in dairy animals. Journal of arthropod-borne diseases, 10(2): 258.

Talkhan, O., M. Radwan and M. Ali, 2010. Cattle babesiosis and associated biochemical alteration in *Kalubyia governorate*. Nature sciences, 12: 24-27.

Tufani, N., M. Fazili, H. Malik, S. Beigh and K. H. Dar, 2015. Clinico haematological profile and therapeutic management of acute babesiosis in a holstein-friesian crossbred cow. Veterinary clinical science, 3(3): 11-14.

Ziapour, S. P., B. Esfandiari and M. R. Youssefi, 2011. Study of the prevalence of babesiosis in domesticated animals with suspected signs in Mazandaran province, North of Iran, during 2008. Journal of animal and veterinary advances, 10(6): 712-714.

Zulfiqar, S., S. Shahnawaz, M. Ali, A. M. Bhutta, S. Iqbal, S. Hayat, S. Qadir, M. Latif, N. Kiran and A. Saeed, 2012. 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(2): 104-108.