

Effects of Babesiosis on Blood Hematological and Biochemical Parameters of Cows

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ABSTRACT

Background: Babesiosis is a life-threatening disease caused by a parasite of the *Babesia* spp., This infects and destroys the Red blood cells. The disease is transmitted mainly by ticks which become infected by feeding on infected cows. The animals will face certain death if the disease is not properly treated on time. Due to this disease the livestock industry suffers many problems and has faced many challenges and losses. The objective of the current study is to find out the effect of hematological and biochemical parameters of babesiosis on cow blood.

Materials and Methods: The present study was carried out in the summer season of Nangarhar province. A total of 30 cows of Watani and 30 cows of Crossbred were selected. The cows were then divided into 15 babesia- infected and 15 healthy cows as controlled groups for both breeds. The sample for blood was collected from the jugular vein of animals using an EDTA tube which contains 2ml of blood for biochemical test.

Findings: The obtained results showed that hematological parameters such as Red blood cells and Hemoglobin in Watani and Crossbred cows were significantly decreased ($P \leq 0.05$) in babesia-infected cows than healthy controlled group. While the number of White blood cells was significantly higher ($P \leq 0.05$) in babesia- infected cows. In the case of biochemical parameters, the amounts of Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) both in Watani and Crossbred cows were significantly higher ($P \leq 0.05$) in babesia-infected cows.

Conclusion: This study concluded that the amount of total protein was significantly decreased ($p \leq 0.05$). The amount of cholesterol remained significantly higher. Both hematological and biochemical parameters were significantly different in babesia-infected cows than in controlled healthy cows in the present study.

Keywords: Babesiosis, cows, hematology, biochemical factors.

INTRODUCTION

The cattle industry can be overwhelmed with plentiful diseases, among them the tick-borne ailments are of considerable importance. These diseases have a negative influence on animal health and consequently the country's economy (Mghirbi et al., 2008). Bovine babesiosis is an important tick-borne disease all over the world but commonly in subtropical and tropical regions (Sharma et al., 2016). *Babesia* is an intraerythrocytic hemoprotozoan affecting animal Red blood cells (Zint et al., 2003). Blood parasites have recently been under investigation, strong accumulated evidence was found to link the resulting anemia with oxidative damage because of lipid peroxidation of Red blood cells (Nazifi et al., 2011).

Babesia is the second most common parasite found in mammals blood after trypanosomes (Yabsley & Shock, 2013). Therefore, the climatic conditions are favorable to the multiplication and tick growth which are the most important ectoparasites of livestock equally in tropical and sub-tropical countries (Ibrahim et al., 2012). Ticks not only cause direct losses by sucking the blood of the host animal, but they are also responsible for several blood-borne diseases such as anaplasmosis, babesiosis and theileriosis (Durrani et al., 2008). Hematological and Sero-biochemical changes are the indicators of disease severity and are considered to be good tools for the diagnosis, prognosis and for the effective treatment (Nazifi et al., 2010).

Hematological and Biochemical parameters are both very essential almost to diagnose the disease, monitor the disease course, and follow up the response to treatment. Data regarding the pathogenesis and prognosis of the disease can be obtained from these parameters (Sevinc et al., 2007). The present study was conducted to determine the effect of Babesiosis on Hematological parameters (Red blood cells, White blood cells and Hemoglobin) as well as the effects of Babesiosis on Biochemical parameters (Cholesterol, Total Protein, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) in Jalalabad city, eastern zone of Afghanistan.

MATERIALS AND METHODS

Study period and study Area

The prevalence of Babesiosis is high in all seasons of Nangarhar province, especially in the hot summer season. Therefore, this research was conducted in the Central Animals Clinic in Jalalabad city which is located (34.26° N, 70.26° E) The study was performed during June 2020 to August, 2020 for three months period. Daytime maximum average temperature was recorded around 40°C (104°F).

Selection of Animals

A total 60 cows were selected, 30 cows from the local (Watani) breed and 30 cows of Crossbred were studied. Each breed was then divided into two more groups, 15 cows infected with Babesia and 15 healthy cows as the (control) group. Blood samples were collected from sick animals at the Central animal clinic of Nangarhar province. Blood samples of healthy cows were collected from Hada cow's farm and other samples were collected from Surkhroud district.

Methodology

For the diagnosis of cows infected with Babesia, a sample was taken from the ear, the hair was removed with scissors, then the area was cleaned with an antiseptic and the ear vein was punctured with a needle and a drop of blood was taken from it, and then the blood was put on the slide and covered by another slide. The said slide was air-dried and fixed with 95% Methanol for 1 minute. After that, it was stained with 10% of Giemsa for 20-30 minutes. After staining, the slide was then washed with clean water and dried in the open air. The slides were further examined under a microscope (Model 222041, Labophot Nikon, Japan with 100× magnification) for the diagnosis of Babesiosis, the procedure was described by (Hendrix & Robinson, 2022).

Sampling

10 ml of Blood samples were taken from the animal's jugular vein and divided into two tubes. The tube containing anti-clotting materials as (ethylene diamine tetra-acetic acid) for the analysis of blood parameters. The number of Red blood cells and White blood cells and, the amount of Hemoglobin have been determined.

For the analysis of biochemical parameters, the blood was put into tubes that did not contain anti-clotting agents, finally total protein, cholesterol, AST and ALT were measured. The procedure for its determination was completed.

Procedure

The number of cells: The number of (RBCs and WBCs) would be determined by the Neubauer haemocytometer (Model No 68052-14 EMS catalog, Hatfield, PA, USA). The method was done in one microliter of blood with 30×70mm and 4mm thickness.

Total White blood cells count. : Total White blood cells count (WBCs), were estimated by hemocytometer using Turke's solution 500 ml as diluting fluid (Azer Scientific Inc. India) The number of White blood cells was done under the microscope (Model 222041, Labophot Nikon, Japan with 100× magnification) for the diagnosis of Babesiosis with the help of a Haemocytometer. This procedure was described by (Schalm et al., 1975).

Total Red blood cells count: Total Red blood cells (RBCs) were estimated by Improved Neubauer double hemocytometer, ((Model No 68052-15 EMS Catalog, Hatfield, PA, USA) the Red cells diluted using isotonic Hayem's (RBC) solution (Microxprees, Tulip Diagnostic Company, Goa India). This procedure was also described by (Schalm et al., 1975).

Hemoglobin level: The hemoglobin concentration (Hb) was estimated by the acid hematin method. The hemoglobin level was determined by hemometer in grams per deciliter. The hemoglobin concentration (g/dl) was determined in the method described by (Kadhem, 2016).

Biochemical parameters: Total protein and cholesterol level were both determined by Biuret reaction method using the commercial protein kit (AMEDA Labordiagnostik GmbH, Krenngasse 12, 8010 Graz, Austria). The amount obtained was expressed as (g/dl) (Khan et al., 2012).

Determination of AST and ALT: AST and ALT are the primary application of serum measurement which can detect different etiologic diagnosis of hepatic disease. AST and ALT help identify toxins in the liver, liver disease or liver damage. They are important liver enzymes involved in amino acids metabolism. Biochemical serum analysis of ALT, AST was estimated by Spectrophotometer (Model U2020 Geesthacht, Germany) using commercial chemical kits supplied by Randox LTD (Korea). The result was obtained using by Micro-lab (Biochemistry Analyzer, Q-Line Biotech, India) and the procedure was described by (Anwar et al., 2005).

Statistical Analysis

The obtained data was analyzed by paired t-test using SPSS-10 software (SPSS, 1999) was used for data analysis using guidelines provided by (Steel and Torrie, 1980). The change between the data was shown with a P-value and a significant change (significance) was shown. P-value was mentioned to determine the significance of significant change.

RESULTS

Babesiosis disrupts the hematological profile of local cows

To diagnose this disease, 2 ml of blood samples were collected from the jugular vein using an EDTA tube from local cows under sterile conditions and sent to the laboratory. After the result analysis, we found that the White blood cells in Babesia-infected cows increased significantly ($P < 0.05$) compared to healthy cows. The

hemoglobin and erythrocyte levels decreased significantly ($P < 0.05$) in Babesia-infected cows compared to healthy ones. Briefly, it can be said that cows infected with Babesia, showed an increase in White blood cells and a decrease in hemoglobin level and serokaryotes (Figure 1).

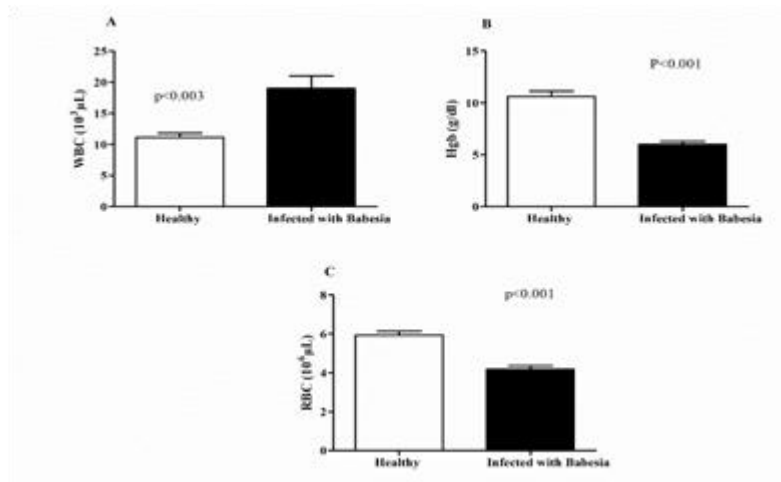


Figure 1. Blood hematological factors in local cows infected with Babesia: (A) White blood cells (B) Hemoglobin and (C) Red blood cells. The result showed a significant decrease ($P < 0.001$) in White blood cells and Hemoglobin levels in Babesia-infected local cows. There was a significant increase ($P < 0.003$) in cell size.

Babesiosis disrupts the biochemical profile of local cows

To diagnose this disease, 2 ml of blood sample was collected using an EDTA tube under sterile conditions. After the biochemical tests, the results indicated the levels of AST and ALT in the blood serum increased significantly ($P < 0.05$) in cows infected with Babesia compared to healthy cows. The amount of total protein decreased significantly ($P < 0.05$) in Babesia-infected cows compared to healthy ones. The cholesterol level in cows infected with Babesia was higher than that of healthy cows, but there was no significant increase. There was no significant change in the level of cholesterol. Briefly, it can be said that there was a significant increase in the levels of AST, and ALT and a significant decrease in total protein levels, but there was no significant change in cholesterol level (Figure 2).

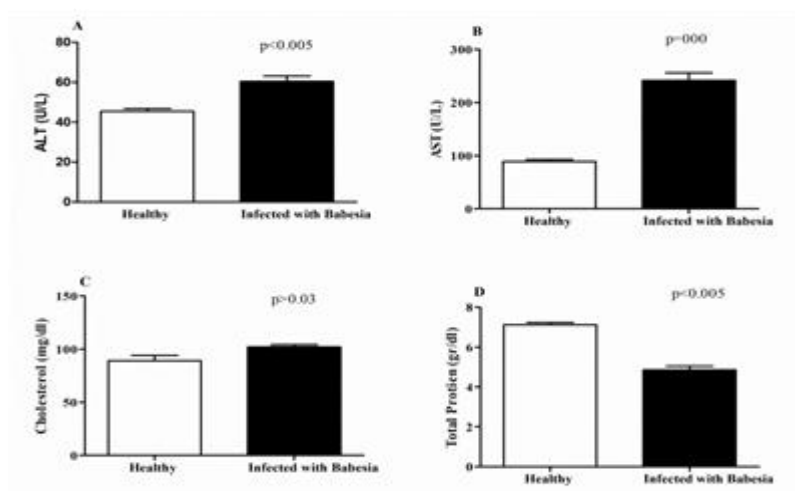


Figure 2: Blood biochemical factors in local cows infected with Babesia. (A) ALT, (B) AST, (C) Cholesterol and (D) Total protein. The results indicated that local cows infected with Babesia showed a significant ($P < 0.05$)

increase in ALT, AST and Total protein levels. There was a significant decrease but no difference in cholesterol level between healthy and cows infected with Babesia.

Hematological profile in Babesia-infected Crossbred cows

After the data analysis of laboratory tests, the results indicated the number of White blood cells increased significantly ($P < 0.001$) in the Cross-bred cows affected by Babesiosis compared to the healthy Cross-bred cows. Hemoglobin and erythrocyte levels were significantly ($P < 0.001$) and ($P < 0.004$) lower in Babesia-infected cows than the healthy cows. The result showed that there was a significant increase in the number of white blood cells and a significant decrease in the hemoglobin and serum level (Figure 3).

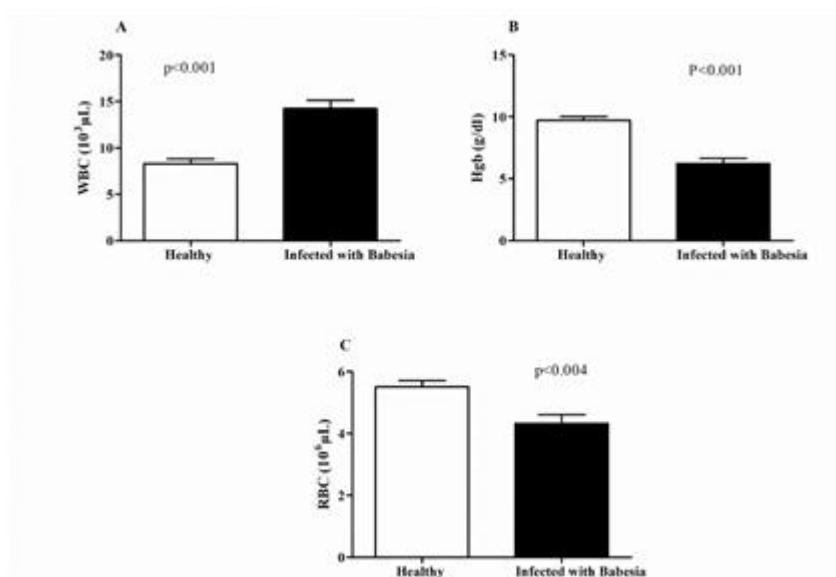


Figure 3. Study of blood hematological factors in Babesia-infected Crossbred cows. (A) White blood cells (B) Hemoglobin and (C) Red blood cells. The results showed a significant ($P < 0.001$; $P < 0.004$) decrease in White blood cells and Hemoglobin level in Babesia-infected crossbred cows, respectively but in healthy animals a significant ($P < 0.001$) increase was seen in the number of White blood cells.

Biochemical profile in Babesia-infected Crossbred cows

Due to the effects of babesiosis, blood samples were collected from the jugular veins of the crossbred cows. Analysis of data from laboratory tests showed that the ratio of AST and ALT levels in the blood serum of Babesia-infected cows had significantly increased ($P < 0.001$) than healthy cows. The result indicated that Babesiosis affected the liver of cow so there was an increase in the liver indicators. However, the level of total protein in Babesia-infected cows was significant compared to healthy cows ($P > 0.05$). There was not seen any significant difference in the cholesterol level between Babesia-infected and healthy cows. In short, it can be said that AST and ALT levels increased significantly in Babesiosis (Figure 4).

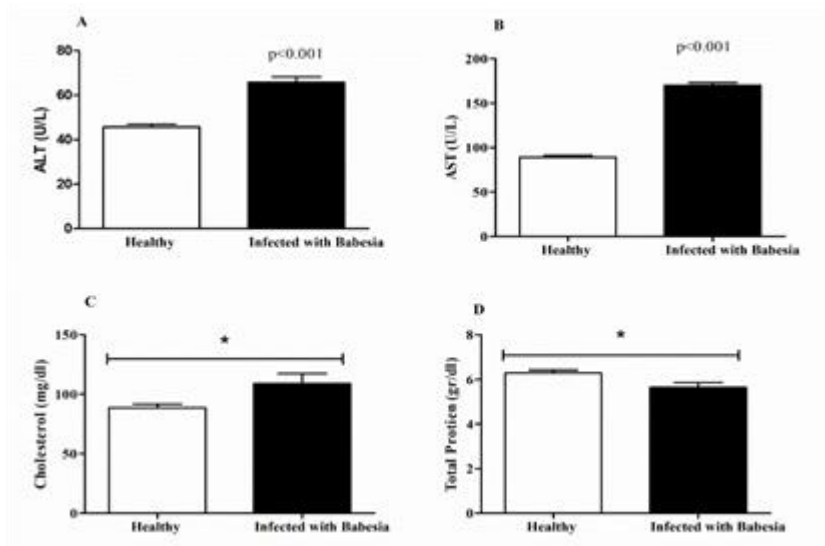


Figure 4. Study of blood biochemical factors in Babesia-infected crossbred cows. (A) ALT (U/L), (B) AST (U/L), (C) cholesterol and (D) total protein. The results showed a significant increase ($P < 0.05$) in ALT and AST levels of Babesia-infected cows, but a significant change was not found in total protein and cholesterol levels.

DISCUSSION

In the present study, a significant decrease in the number of White blood cells (WBCs) compared to healthy cows was seen in Fig 1. This result was in accordance with the result of (Mohamed Saied, 2017) that approximately confirms our current result. During biochemical tests, a significant increase was found in Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) levels in the blood serum of Babesia-infected cows compared to healthy cows. There was a significant decrease in serum protein levels in cows suffered from Babesiosis compared to healthy cows (Fig 2). A significant increase in AST and ALT levels might represent the harmful effects of Babesia toxic metabolites. The hematological test is widely used as a source of information on the disease status, activity and related treatment (Mahmood, 2014). The results of our research showed a significant decrease in Red blood cells (RBCs) and Hemoglobin (Hb) levels in cows infected with Babesiosis compared to healthy cows, but a significant increase was seen in the number of White blood cells (WBCs) (Fig 3). The present result confirms the finding of (Mahmood, 2013) which was done in Egypt on buffaloes.

A significant decrease in Red blood cells, Hemoglobin and Blood platelets shows the presence of macroscopic hypochromic anemia, which was considered one of the important clinical signs of anemia in animals suffering from Babesiosis (Wright & Mermin, 1989). The Babesia parasite can destroy Red blood cells. Due to changes in Red blood cells, osmotic breakdown of Red blood cells occurs, Red cells are easily destroyed and consequently, hemolysis occurs (Kakoma et al., 1984). During Babesiosis, the secretion of liver enzymes increases therefore, the liver is destroyed (Sharma et al., 2016), and the parasites in the blood indirectly cause liver dysfunction (Alam et al., 2011). The result of the present study showed a significant increase in the biochemical parameters of AST and ALT in the blood serum of cows suffered from Babesiosis compared to healthy cows, and a slight increase was seen in cholesterol, but the total protein was the opposite. There has been a significant decrease (Fig 4). It is clear from the study that our result confirms the result of many other

researchers (Salem et al., 2015), (Wright & Mermin, 1989), (Kakoma et al., 1984). Therefore, for more clarification, we could say that Leukocytosis and Monocytosis in animals infected with Babesia, from the clinical observation, it was recognized that the protozoa can stimulate the lymphoid system and bring an increase in White blood cells during the disease (Ibrahim et al., 2012), and significantly reduced total protein and albumin levels in Babesia-infected calves. Babesia can interfere with liver function, decrease albumin synthesis and affect total protein levels (Werner et al., 2004).

It remains to be seen that Babesia parasites are found all over the world on a large scale, destroy the cells, cause anemia, jaundice and hemoglobin deficiency (hemoglobinuria) and have significant global economic effects in the fields of Veterinary medicine and Medicine. The serum concentrations of ALT and AST are called indicators of liver function. Higher levels of AST and ALT concentrations were caused by changes in liver function (Alam et al., 2011). As a result, the effects of liver and kidney can be seen in animals suffering from Babesia disease (Aziz et al., 2020). The present study has the similarity and confirms the results of those researches who have studied the hematological and biochemical parameters in Cows infected with babesiosis in tropical and sub-tropical regions of the world (Hussein et al., 2007).

CONCLUSION

Babesiosis is a protozoan disease caused by the babesia parasite inside Red blood cells. It is transmitted from one animal to another by ticks, if the proper treatment of infected animals is not done on time. This disease can cause great economic losses in the cattle industry. Compared to healthy cows, a significant decrease was seen in the number of Red blood cells and Hemoglobin level in local cows and crossbred cows infected with Babesia, and vice versa. There was a significant increase in the number of White blood cells. The blood serum biochemical tests in the babesia-infected cows showed a significant increase in AST and ALT compared to healthy cows in contrast. There was a significant decrease in the total protein level. There was a slight increase in the cholesterol level, but it was not significant. There was a significant difference in the hematological and biochemical factors in Babesia-infected cows compared to healthy cows.

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