

Research Article

Assessment of lipid and thyroid biomarkers in cattles infected with babesiosis in Lahore, Pakistan

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Citation

Mariyum Hussain, Hamida Ali, Nabila Roohi and Muhammad Naeem. Assessment of lipid and thyroid biomarkers in cattles infected with babesiosis in Lahore, Pakistan. Pure and Applied Biology. Vol. 10, Issue 1, pp311-317. <http://dx.doi.org/10.19045/bspab.2021.100033>

Received: 16/06/2020

Revised: 24/08/2020

Accepted: 04/09/2020

Online First: 21/09/2020

Abstract

Babesiosis is a tick-borne disease found in cattles, transmitted by piroplasm which enters into the host RBCs and destroys them completely. It also affects different organs resulting into the death of animals. This study was aimed to evaluate the biochemical and hormonal outcomes of babesiosis in cattles in Lahore, Pakistan. Blood samples were collected from 89 cows from military farm of Lahore and brought to physiology/endocrinology laboratory, University of Punjab for biochemical analysis. Different parameters such as glucose, cholesterol, high density lipoprotein (HDL) and triglycerides (TG) were analyzed by chemistry analyzer while thyroxine (T4) was determined by enzyme-linked immunosorbent assay (ELISA). Statistical analysis was carried out using independent samples t-test. Out of 89 cattles, 25% (n=22) were found to be infected with *Babesia*. Results of present investigation demonstrated prominent increase in levels of glucose ($P<0.001$) (41%) and triglycerides ($P<0.001$) (22%) in infected cattles. Whereas, significant decrease was evidenced in concentration of thyroxine ($P<0.01$) (21%) and HDL ($P<0.001$) (30%) in *Babesia*-infected cattles as compared to non-infected cattles. However, levels of cholesterol demonstrated non-significant decrease (15%) in *Babesia*-infected animals than healthy animals. Such variations in thyroid and lipid profile can increase the risk of hyperglycemia, hepatic and cardiac dysfunction among cattles.

Keywords: *Babesia*; Cattles; ELISA; High density lipoprotein; Thyroxine; Triglycerides

Introduction

Babesiosis is a tick-borne hemoprotozoan disease with host-specific features carried by members of the *Babesia* genus. Babesiosis affects cattle, horses, sheep, goats, pigs, dogs and wild animals. Life cycle of *Babesia* within the tick body comprises of three stages i.e. gamogony, sporogony and merogony. *Babesia bovis*, *Babesia bigemina* and *Babesia divergens*

are the parasites which are responsible for babesiosis in cattles. However, cattles are affected more by *B. bovis* and *B. bigemina*. *Rhipicephalus microplus* and *Rhipicephalus annulatus* are the ticks which are responsible for the transmission of *B. bovis* and *B. bigemina* among cattles [1].

The location of *B. bovis* is in the center of erythrocyte and is considered as a small

parasite. It measures about 0.5-1.0 μm wide and 1-1.5 μm long. While, *B. bigemina* is pear-shaped, large parasite which is about 1-1.5 μm wide and 3-3.5 μm long and with a two different red-staining dots (*B. bovis* and *B. divergens* have one only) [1].

The acute disease generally runs a course of one week. The first sign is fever, which persists throughout the course of disease, and is accompanied with inappetence, increased respiratory rate, muscle tremors, anemia, jaundice, weight loss, hemoglobinemia and hemoglobinuria in the final stages. Cattles infected with babesiosis have reduced fertility, increased fatality rates and heightened risk of producing spoilt milk. Adult female ticks also cause problems in cattles in gaining weight [2].

Infected animals should be treated with anti-parasitic drugs. The proper administration and efficacy of drug can lead to the successful treatment only if the disease is diagnosed earlier [3]. The infection is prevalent in tropical and subtropical areas. Babesiosis is more common in the wet season when the tick vectors are abundant, but less common in dry weather or dry areas where the survival of ticks is limited [1].

Glucose is produced by the liver through the process of glycogenolysis and gluconeogenesis [4]. An elevated level of glucose in the blood predisposes individuals to certain metabolic diseases.

The liver failure in cattles infected with *Theileria* and *Babesia* species is due to decreased levels of globulin and albumin which causes a decrease in the total protein [5]. Due to decrease in the albumin synthesis and increase in the albumin catabolism, the previous studies showed that theileriosis and babesiosis have a damaging impact on hepatocytes which thus causes complication in the hepatic function [6]. Liver produces cholesterol which is important for the body function. Excess cholesterol will increase the risk of a heart diseases in cattles [7]. High density

lipoprotein (HDL) and low density lipoprotein (LDL) are the two main types of cholesterol [7, 8].

Thyroid, an endocrine gland produces two hormones: T4 (tetraiodothyronine/thyroxine) and T3 (triiodothyronine). These hormones regulate the different processes of metabolism [9-11]. T4 formed by the thyroid gland is carried to the tissues for modification by different binding proteins. T4 through different courses of de-iodinases is converted to T3 [12].

The present study was aimed to determine the alterations in thyroxine hormone, glucose level and lipid profile (cholesterol, HDL and TG) in cattles due to babesiosis.

Materials and Methods

Sample collection

A total of 89 blood samples were collected from cows from military dairy farm in Lahore, Punjab and were brought to physiology/endocrinology laboratory, Department of Zoology, University of the Punjab, for further biochemical and hormonal analyses.

Microscopic examination of *Babesia*

The blood sample of each animal was smeared on glass slide for microscopic examination. Prior to examination, smeared slides were air-dried quickly and fixed with methyl alcohol for one minute. After fixation each slide was again air-dried and stained with Giemsa solution for 30-40 minutes. After staining, slides were allowed to dry and washed with distilled water so that any residue left could be washed away. The stained slides were examined under the microscope using immersion oil.

Biochemical analysis of blood samples

Blood samples collected in vacutainers were centrifuged for 5 minutes and serum was separated from the samples and then stored at -80°C for the biochemical and hormonal analyses. Blood glucose, cholesterol, HDL and triglycerides were analyzed by commercially available kits using clinical chemistry analyzer.

However, thyroxine was analyzed by ELISA.

Statistical analysis

Data was analyzed using Graph Pad Prism (USA) software. Independent samples t-test was applied to find out significant differences in the different biochemical and hormonal parameters between the *Babesia*-infected cattles and the non-infected ones.

Ethics statement

The study was approved by the institutional review committee of University of Punjab, Lahore, Pakistan.

Results

Overall 25% (22/89) cattles were found to be infected with babesiosis. Table 1 shows the means of different biochemical and hormonal parameters in infected and healthy cattles. The mean glucose level was 48.22 ± 2.80 mg/dL in healthy cattles and 67.96 ± 3.26 mg/dL in infected cattles. Marked increase of 41% ($P < 0.001$) in the glucose level was detected in infected group as compared to healthy group of cows (Fig. 1a). Moreover, the mean T4 values of healthy and infected animals were 200.3 ± 8.55 ng/mL and 158.6 ± 16.14 ng/mL, respectively. A significant decrease of 21% ($P < 0.05$) in the T4 level was observed in infected group as compared to healthy group of cows (Fig. 1b).

The mean cholesterol levels of healthy and infected animals were 106.7 ± 6.01 mg/dL and 92.32 ± 5.09 mg/dL, respectively. Cholesterol level demonstrated slight

decrease of 15%, in infected group as compared to healthy group of cows (Fig. 1c). Moreover, the mean HDL values of healthy and infected animals were 62.74 ± 2.66 mg/dL and 44.08 ± 3.70 mg/dL, respectively. A prominent decrease of 30% ($P < 0.001$) in HDL was evidenced in infected group as compared to healthy cows (Fig. 1d). The mean triglycerides values of healthy and infected animals were 40.42 ± 0.73 mg/dL and 49.28 ± 2.67 mg/dL, respectively. A significant increase of 22% ($P < 0.001$) in triglycerides, was detected in infected group as compared to healthy group of cows (Fig. 1e).

Discussion

Babesiosis additionally alluded to as piroplasmiasis, tick fever, red water or texas fever is a risky, obtrusive, febrile and tick-borne disease [13, 14]. The protozoan *Babesia* includes *B. bovis*, *B. bigemina*, *B. major* and *B. divergens*. Among these species *B. bovis* and *B. bigemina* are the most crucial species that cause babesiosis in cows worldwide [15, 16]. Ticks belonging to species of *Ixodid*, *Rhipicephalus*, *Haemaphysalis* and *Dermacentor* affect cows in Pakistan [17]. According to Pakistan Economic Survey (2013-2014), it is estimated that the population of cows are from 36 to 41 millions in Pakistan. Cows are the main source of milk giving 19,000 to 32,000 million tons milk every day. The economy of Pakistan thus depends upon its livestock.

Table 1. A comprehensive presentation of biochemical and hormonal parameters in *Babesia*-infected and healthy cattles

Parameters	Healthy Cattles	Infected Cattles	P-value	%↑or↓
Glucose (mg/dL)	48.22 ± 2.80	67.96 ± 3.26	0.0001***	41% ↑
Thyroxine (T4) (ng/mL)	200.3 ± 8.55	158.6 ± 16.14	0.0160*	21% ↓
Cholesterol (mg/dL)	106.7 ± 6.01	92.32 ± 5.09	0.1610	15% ↓
HDL (mg/dL)	62.74 ± 2.66	44.08 ± 3.70	0.0002***	30% ↓
Triglycerides (mg/dL)	40.42 ± 0.76	49.28 ± 2.67	< 0.0001***	22% ↑

Values are mean±SEM, ↑ or ↓ indicates increase or decrease, respectively
 *and ***indicates significance at $P < 0.05$ and $P < 0.001$, respectively

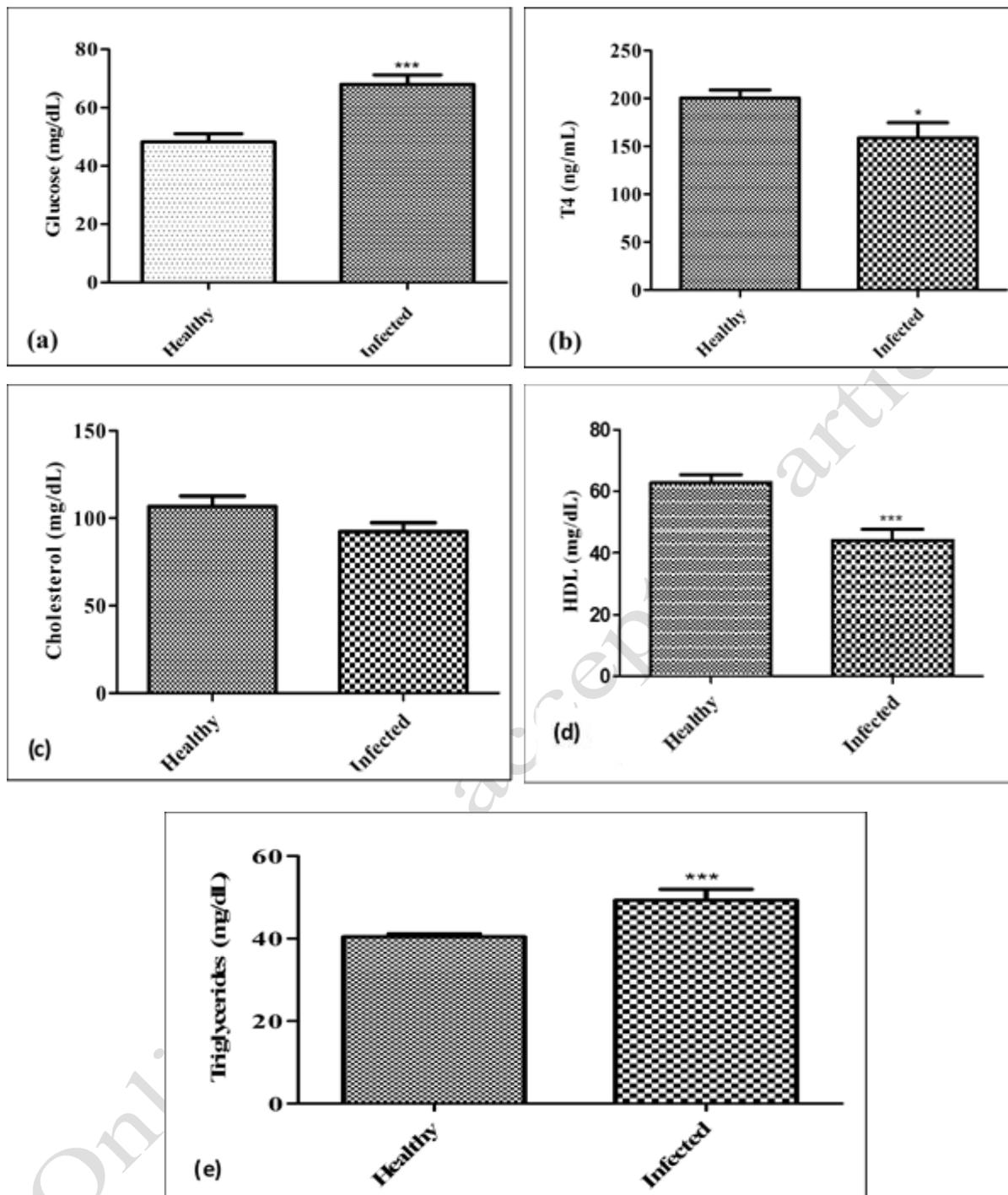


Figure1(a-e). Graphical representation of different biochemical and hormonal parameters in *Babesia*-infected and healthy cattles. (Values are Mean \pm SEM, Significant at * $P \leq 0.05$ and *** $P \leq 0.001$)

Overall the prevalence of babesiosis was recorded to be 25% among cows in this investigation. Different parameters like glucose, thyroxine, cholesterol, HDL and triglycerides were determined. Cattles

infected with *Babesia* parasites showed significant increase in the level of glucose i.e 41%. The findings of this investigation were supported by [18-21]. It is well known that increase in gluconeogenesis

causes increase in the level of blood glucose [22].

Significant decrease in the thyroxine concentration has been observed in our study. The secretory product of thyroid gland is thyroxine hormone (T4) [23]. The presence of thyroxine in animal's blood is the indicator of iodine availability [24]. Decrease in the T4 concentration is due to deiodination of T4 to T3, a more active form which results due to high metabolic load [23].

In our study significant decrease in the serum cholesterol has been observed. Similar finding has also been reported by [25]. According to [26] diarrhea and anorexia may be one the reason for decreased level of cholesterol. We found a significant decrease in HDL level. [27] also reported reduction in the concentration of HDL. Decrease in HDL level may be due to decreased level of cholesterol [28]. Increase in the hepatic production of triglycerides or abnormality in the removal of triglycerides from circulation causes an increase in the level of triglycerides. Moreover, in acute phase the increased triglyceride level are thought to be associated with host response [29].

Blood chemistry is changed in different conditions, for example, extraordinary irresistible ailments, liver problems, tissue injury, and numerous other physiological issues. Hence, biochemical analyses of blood serum are very useful to get insight into the metabolic and health status of animals. Biochemical determination of serum constituents can provide important information as relating to age, sex, nutrition, and physiological status of the animal [30]. Early prediction of potential health risks associated with babesiosis will be helpful in improving the health of these animals by timely treatment and management. Timely and accurate treatment measures will no doubt improve the outcomes of treatment thus increasing the survival rate.

Conclusion

In conclusion, the results of present study suggested that cows infected with babesiosis are facing stress situation and are at increased risk of hyperglycemia and consequent diabetes as indicated by high blood glucose. Further, raised levels of triglycerides but decreased HDL indicated that these cows were also at increased risk of hepatic and cardiac dysfunction.

Authors' contributions

Conceived and designed the experiments: N Roohi, Performed the experiments: M Hussain, Analyzed the data: H Ali & M Naeem, Contributed reagents/ materials/ analysis tools: M Naeem & N Roohi, Wrote the paper: M Hussain & H Ali.

Acknowledgements

The authors wish to thank Lt.Col Mohammad Hasan for his continuous support and help throughout their research.

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Online first accepted article