Int.J.Curr.Microbiol.App.Sci (2020) *9*(8): 299-306

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 8 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.908.035

Study of some Hematological and Biochemical Parameters of Sheep Babesiosis in Shikan Locality – North Kordufan State –Sudan

A. H. Abdalla[1], M. I. M Fangama[2*], S. E. Suliman[1] and A. M. Abdedalla[1]

[1]College of Veterinary Medicine and Surgery, Sudan University of Science and Technology, Khartoum, Sudan
[2]Ministry of Health, Qatar Public Health Department

**Corresponding author*

Keywords

Hematological and biochemical parameters, Babesiosis, Sheep

Article Info

Accepted:
10 July 2020
Available Online:
10 August 2020

A B S T R A C T

The aim of this study was to evaluate some hematological and biochemical parameters of ovine babesiosis from March 2018 to January 2019 in Shikan Locality North Kordufan State. A total of 150 samples of whole blood and blood for serum were taken from the jugular vein of naturally suspected sheep using visual inspection and clinical examination of heart rate, respiratory rate and body temperature. For identification of the parasite Giemsa - stained blood smear method was used. Also hematological (hemoglobin concentration, red blood cells, hematocrit, red cell distribution width, platelet distribution width and plateletcrit) and biochemical (Total protein, albumin, urea, creatinine, cholesterol, and triglyceride) parameters were measured. The results revealed that all health parameters were elevated in infected animals (20 animals) compared with negative infected animals (76 animals) and control animals (54 animals) to ovine babesiosis. There was decrease of hemoglobin concentration (4.83±0.87),

Introduction

Babesiosis is a group of tick borne diseases caused by several species of protozoa of the genus Babesia. These organisms are capable of infecting all species of domestic animals, and also found in some wild animals, which serve as reservoir of infection (Losos, 1986). Sheep and goats are infected by Babesia motasi and *Babesia ovis* . Sheep babesiosis is of considerable economic importance in the areas are infected with *Rhipicephalus burse* (Radostits *et al.*, 2007).

During infection with *Babesia*, the release of pharmacologically active substance and destruction of erythrocytes play a major role in the parasitemia of the disease. However,

the propionate role of each varies with the individual species of *Babesia* (Soulsby, 1982). Anemia is associated with emergence of the parasite from red cells open however erythrocytes loss is attributed to the mechanical rupture of cells by parasites although there have been no detailed studies of this in domestic animals (Mohoney, 1977).

Developmental life cycle include merogony, gamogony and sporogony. During tick feeding the infection occurred by sporozoites which invade erythrocytes to divide by binary fission to produce merozoites then to gametocytes which can initiate the infection in tick vector (Melhorn and Piekarsk, 2002).

Diagnosis of the disease clinically by appearance of fever, malaise and restlessness, anorexia and anemia. Icterus, hemoglobinurea and ascites may appear during late stages and progressive debility terminate to death (Smith *et al.*, 1972; Soulsby, 1982) . Breathing is labored and rapid, the heart beat is fast and loud, nervous signs are hyperexcaitability, moving the object , impaired vision and changing of urine to red color (Nyndo, 1992). In ruminants ruminal movement ceased and abortion may occur (Urquhart *et al.*, 1996).

Babesiosis can be confirmed by performing Giemsa stained thin smear or Romanowsky – stained smear (Soulsby, 1982 Urquhart *et al.*, 1996). Serology includes enzyme – linked immunosorbent assay and complement fixation test (Salih *et al.*, 2015).

Molecular methods applied such as probes, polymerase chain reaction, reverse line hot hybridization and real time PCR (Mosqueda *et al.*, 2012). Currently antiprotozoal agents as diminazene aceturate and imidocarb dipropionate were administered (Enbiyale *et al.*, 2018). Main methods for prevention and controlling of *Babesia* are immunization, chemoprophylaxis and vector control (Demessie and Derso, 2015).

Piroplasmosis in the Sudan was early reported in the beginning of the past century. The disease was reported in 1905 and the research was done (Hoogstral, 1956; Abdoun, 1984; Hashim, 1984). The aim of the paper was to evaluate some hematological and biochemical parameters of sheep babesiosis in Shikan Locality.

Materials and Methods

Study area

This study was carried out from March 2018 up to January 2019 in Skikan Locality – North Kordufan State.

Collection of samples

A total of 150 whole blood samples and blood for serum were taken from the jugular vein from naturally suspected sheep by babesiosis after visual inspection and clinical examination of the heart rate, respiratory rate and body temperature (Kelly, 1984; Matijatko *et al.*, 2007).

Laboratory techniques

For identification of the parasite Giemsa – stained blood smear method was used. Also hematological techniques were used for determination of haemoglobin concentration (HGBg/dl), red blood cell counts (RBCs×1012) hematocrit (HCT %) red blood cell distribution width (RDW-SDFL), platelet cell count (PLT ×109/L), mean platelet cell volume (MPV/FL) and plateletcrit (PCT/M/L) (Schalm *et al.*, 1986; Shino *et al.*, 2003). All these hematological parameters were analyzed by automated hematological analyzer (Mindary, 3000). Biochemical analysis of serum(150 samples) for

determination of albumin, globulin, urea, creatinine, cholesterol and triglyceride using kits (Bio system kits 350).

Drugs

Diminazene aceturate was administered at dose rate 2mg/kg b.wt., I/M single dose followed by supportive therapy given based on requirement of the individual case (Vidhya *et al.,* 2011)

Statistical analysis

The results were analyzed by-way analysis of (ANOVAs) followed by pair – wise comparisons using the Duncan test. The computer software SPSS version 17.0 for windows was used for analysis.

Results and Discussion

The suspected sheep [96 animals] were examined clinically for heart rate, respiratory rate and body temperature as shown in table 1. But after microscopic examination of stained smears 20 animals (13.3%) were found infected by *Babesia ovis* (Table 2).

Table 3 showed that the hematological changes in hemoglobin concentration, red blood cell count and hematocrit, and these parameters were decreased in positive infected animals by B.ovis compared with negative non-infected animals by the disease and control animals. But there was slight increase of red blood cell distribution width (19.1±6.37) compared with control animals (18.3±4.05).

Platelet cell counts were decrease in infected animals (Table 3) compared with control animals, while the mean platelet cell volume was slightly increased (7.23±1.94) compared with control animals (6.85±1.38), whereas, plateletcrit was decreased (0.49 ±0.53) in the infected animals compared with control animals (32.9±119). There were lightly changes in mean corpuscular volume compared with control animals (Table 3), but the mean corpuscular hemoglobin concentration was decreased in sheep positive to *B. ovis*.

As shown in table 4, total protein and globulins were increased, while albumin was decreased in infected animals. Also urea and creatinine were increased compared with control animals. Cholesterol value was increased in infected sheep, but there was slight increase in triglyceride (Table 4).

Treatment

All sheep infected by *B.ovis* were treated by diminazene aceturate followed by supportive treatment and no death occurred.

Table.1 Estimation of clinical parameters of infected sheep (N=20) and non-infected sheep (N=76) for babesiosis in Skikan Locality – North Kordufan State

Parameter	Infected	Non-infected	Control
Heart Rate/min	114.64±6.19	76.27±11.29	71.71±1.13
Respiratory Rate/min	54.48±2.17	25.87±5.29	23.70±1.10
Body Temperature/c^{o}	40.87±0.89	39.17± 0.14	38.70±1.20

Table.2 Percentage of sheep babesiosis (N=20) in Shikan Locality – North Kordufan State

Variable	Frequency	percent%
Control	54	36.0
Positive	20	13.3
Negative	76	50.7
Total	150	100.0

Table.3 Some hematological value of sheep babesiosis Locality – North Kordufan State

Variable	Mean Std. Deviation Positive	Mean Std. Deviation Negative		Mean Std. Deviation Control
HGB g/DL	4.83±0.87	7.02±0.55		9.11 ±1.24
RBCs×1012/L	3.42±1.10	4.48±1.79		6.42±2.54
HCT%	13.2±5.77	15.4±4.55		21.0±6.55
MCV flu	33.4±2.50	35.2±3.70		33.8±3.78
MCHCg/do	42.1±15.2	49.3±15.3		48.5±21.0
RDW- SD fl	19.1±6.37	17.3±5.69		18.3±4.05
PLTx109/L	346±203	475±317		531±390
MPV fl	7.23±1.94	6.76±080		6.85±1.38
PCT M/L	0.49±053	52.1±182		32.9±119

Table.4 Biochemical values of sheep bebeiosis (N=150) In Shikan Locality – North Kordufan State

Variable	**Mean** **Std. Deviation** **Positive**	**Mean** **Std. Deviation** **Negative**	**Mean** **Std. Deviation** **Control**
Total protein g/dl	8.97±0.80	5.93±092	7.45±0.51
Albumin g/dl	3.19±0.300	3.15±0.77	3.66±1.00
Globulin g/dl	5.40±1.01	3.16±0.97	3.72±1.03
Urea mg/dl	34.5±9.26	25.0±9.92	25.1±8.96
Creatinine mg/dl	1.14±0.41	0.72±0.24	0.83±0.25
Cholesterol mg/dl	55.0±16.26	48.0±12.9	50.2±9.15
Triglyceride mg/dl	59.9±18.99	57.3±7.83	58.1±12.7

According to the present study, different changes due to parasitemia were observed in the infected sheep. The observations were in accordance with the findings by Razmi *et al.*, (2003); Aktas and Altay. (2007) and Sevine *et al.*, (2007). Decrease in red blood cells count, hematocrit, mean cell volume and hemoglobin concentration levels in infected sheep. These results were consistent with previous findings by Voyvoda *et al.*, (1997) and Hadadazadeh *et al.*, (2002). In addition decline in hemoglobin concentration and red blood cell count observed in other studies that was previously performed on

clinicopathological changes induced by *B.equi* and *B. gibsoni* (Ambawat *et al.,* 1999; Trotta *et al.,* 2009). The present of anemia may be attributed to immunomediated phenomena by auto antibodies directed against component of membrane of infected and uninfected erythrocytes (Rubino *et al.,* 2006).

Production of toxic hemolytic factors of the parasite due to (Rafaj *et al.,* 2007) mechanical damage by trophozoite causing intra-erythrocytic binary fission (Zobba *et al.,* 2008), erythrophagocytosis and through releasing vasoactive molecules such as kallikein (Soulsby, 1982; Brockus and Andresen 2003). Concerning the erythrocyte indices, with parasitemia rates progression, a decrease was observed in level of mean cell volume and mean corpusular hemoglobin concentration. As parasitemia increased, a depletion in mean cell volume and mean corpuscular hemoglobin concentration was evident that indicated microcytic-hypochromic anemia. The result was in accordance with reports of Uilenberg (2006) Rubino *et al.,* (2006), and Zobba *et al.,* (2008) who recorded microcytic-hypohromic anemia in horse infected with *B.equi*.

On the other hand polychromatophilic erythrocytes (Synonymous reticulocytes) in blood smears pointed out a hemolytic anemia. Reduction in mean cell volume level may be due to decrease in hematocrit level and that attributed to the dilution of blood and subsequently mean cell volume. Also the most common abnormality of erythrocytes parameters is anisocytosis which was detected in infected animals and in reference to the value of mean cell volume which was below the normal values associate with spherocytosis (Zygner *et al.,* 2007). Polychromatophilic erythrocytes have a deficient component of hemoglobin concentration, therefore, the mean corpuscular hemoglobin concentration decreases in ovine/caprine bebesiosis (Brockus and Andresen, 2003) as parasitemia of disease increased. The destruction of circulating red cells by auto antibodies is directed against infected and non-infected red cell membranes resulting in intravascular and extravascular haemolysis (Day, 1999; Irwin, 2005). However, Toboada and Lobetti (2005) proposed that direct parasitic damage contributes to anemia. Nevertheless, induction of serum hemolytic factors increased erythrophagocytic activity of macrophages and damage inducted by secondary immune system after the formation of antierythrocyte membrane antibodies which prove the importance of the pathogenesis of anemia.

Platelet crit represents the percent of blood volume occupied by Platelets. It is well known that the surfaces of cells are essential for clotting reactions to take place (Khandekar *et al.,* 2006. Generally, Platelet indices could provide valuable information about the nature of thrombocytopenia, and that more attention should be paid to these indices in the diagnosis of thrombocytopenia. Despite the valuable information given by thrombocyte indices. *Babesia* initiates a mechanism of antibody – mediated cytotoxic destruction of circulating erythrocytes (Furlanello *et al.,* 1991).

In this study there was elevation of total protein, urea, creatinine, cholesterol and triglyceride level. The results are in consistent with findings by other researchers (Yeruham *et al.,* 1998; Rahbari *et al.,* 2008; Crongaj, 2010). It is known that renal involvement occurs in *B.ovis* infection (Habella *et al.,* 1991; Uilenberg, 2006) causing elevation in total protein, urea and creatinine level and might have resulted from kidney dysfunction (Uilenberg, 2006) muscle catabolism (Yeruham *et al.,* 1998) and colonization of *B. ovis* in the renal blood circulation (Habella *et*

al., 1991). It is suggested that in ovine babesiosis many potential factors leading to impairment of renal function e.g. acute diffuse proliferative glomerulitis, acute glomerular hemorrhage and acute tubular necrosis (Uilenberg, 2006; Habella *et al.,* 1991). Hypoalbuminemia in current study is in agreement with previous study (Elissalde *et al.,* 1983; Trotta *et al.,* 2009). Reduction of albumin level probably corresponds to disturbance in liver function, urinary loss of albumin associated with renal failure (Proteinuria) and anorexia in relation to high rise of body temperature. Also similar results have been reported previously (Irizary-Rovira *et al.,* 2001. Diana *et al.,* 2007) concerning the increase of total protein and globulins levels, these findings in all accordance to studies of Camacho *et al.,* (2005); Rubino *et al.,* (2006); Trotta *et al.,* (2009). Generally the study of hyperproteinemia can be attributed to an increase in the globulin concentration in response to parasitic antigen and released hemoglobin from destructed erythrocytes. Elissalde *et al.,* (1983) and Camacho *et al.,* (2005) recorded the elevation in cholesterol and triglyceride concentration that expectable. The slight elevation of cholesterol and triglyceride concentration can be due to liver compensatory reaction to the loss of protein of adipose tissue metabolism during *Babesia* infection (Camacho *et al.,* 2005).

In general, ovine babesiosis causes high morbidity rates among susceptible sheep, but the treatment by diminazene accurate (2mg/kg b.wt) followed by supportive therapy after accurate diagnosis giving successful results (Vidhya *et al.,* 2011; Masqueda *et al.,* 2012).

There was improvement effect after administration of diminazene aceturate (2mg /kg b.wt, I/M single dose with supportive treatment (multivitamins+ i.v.fluids). These results were accordance to Vidhya *et al.,* (2011). Symptomatic therapy along with fluids was given based on requirement of the individual case. Visible improvement efficacy of this drug was observed by negative blood smear examination of infected sheep . In conclusion sheep with babesiosis showed variable depletion in level of hemoglobin, red blood cells, hematocrit, platelets, plateletcrit and mean cell volume in infected sheep babesiosis. In blood chemistry, total protein, creatinine, globulin, cholesterol and triglyceride reveald variable increasament compared to control and negative sheep to babesiosis.

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How to cite this article:

Abdalla, A. H., M. I. M. Fangama, S. E. Suliman and Abdedalla, A. M. 2020. Study of some Hematological and Biochemical Parameters of Sheep Babesiosis in Shikan Locality – North Kordufan State –Sudan. *Int.J.Curr.Microbiol.App.Sci.* 9(08): 299-306.
doi: https://doi.org/10.20546/ijcmas.2020.908.035