

EVALUATION OF SERUM SIALIC ACID LEVEL AND ADENOSINE DEAMINASE ACTIVITY AS A DIAGNOSTIC SIGNIFICAL TEST IN CATTLE NATURALLY INFECTED BY *BABESIA*. SPP

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ABSTRACT

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The aim of the present study was to evaluate the serum concentration of total sialic acid and adenosine deaminase activity and its effect on the severity of the disease in cattle naturally infected by *Babesia*. A total of 50 female cattle (40 female diseased cattle and used as a group 1, and 10 healthy female control cattle (Clinically and laboratory healthy) were used as a group 2. *Babesia* infection was confirmed with Giemsa staining blood film. Infected animal were grouped into mild, moderate and sever cases according to examination the degree of parasitemia. Compared to the control animals (57.6 ± 22.88 mg/dl and $11.07 \pm 44.35 \mu\text{l}$) of total sialic acid, and adenosine deaminase enzyme activity, respectively. There was marked increase in mean total sialic acid and adenosine deaminase were detected. (102.09 ± 3.93 mg/dl and $65.18 \pm 16.30 \mu\text{l}$), respectively. This marked increase without related to the severity of the disease. In diseased ones correlating was not with sialic acid and adenosine deaminase enzyme ($r=0.027p>0.05$). It can be concluded that *Babesia* infection in cattle induced marked increase in the serum level of sialic acid concentration and adenosine deaminase enzyme activity suggesting that sialic acid and adenosine deaminase would indirectly used as an indicator of invasion of the parasite in the host cells.

تقيم مستوى حمض السيالك ونشاط انزيم الادونزين ديميناز في مصل الدم واهميتة التشخيصية في الماشية المصابة طبيعيا بالبابيزيا

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استهدفت هذه الدراسة تقدير تركيز كلا من حمض السيالى الكلى والمرتبب بالدهون ومستوى انزيم الادونزين ديميناز في دم الماشية المصابة طبيعيا بالبابيزيا وقد شملت هذه الدراسة فحص ٤٠ حيوانا من اناث الماشية التي تعاني من البابيزيا وكذلك عدد ١٠ حيوانا سليما ظاهريا ومعمليا واستخدمت كمجموعة ضابطة وجميعها كانت أعمارها بين ٥-٧ أعوام. وقد تم تأكيد الإصابة بالبابيزيا بعمل شريحة دموية مصبوبة بالجمسا وفحصها للتعرف على الطفيل وتقسيم الحيوانات المصابة حسب درجة تواجد الطفيلي في الدم إلى خفيفة ومتوسطة وشديدة الإصابة مع نتائج. ومقارنة الحيوانات السليمة كانت النتائج هي 57.6 ± 22.88 ملجم/ديسلتر و 11.07 ± 44.35 وحدة/لتر لكلا من حمض السيالى الكلى وانزيم الادونزين ديميناز كما في الجدول رقم (٢) إما الحيوانات المريضة موضع الدراسة فكانت كالاتى. 102.09 ± 3.93 و 65.18 ± 16.30 على التوالي. من هذه النتائج فقد لوحظ زيادة مطلقة وغير مرتبطة بشدة المرض حيث لوحظ ان معامل ارتباط حمض السيالى وانزيم الادونزين ديميناز اقل من ٠.٠٥ %، ومن هذا كله يمكن القول بان الإصابة بالبابيزيا في الماشية تودى الى زيادة ملحوظة ومطلقة في مستوى حمض السيالى ومستوى إنزيم الادونزين ديميناز.

Key words: total sialic acid, adenosine deaminase activity, *Babesia*. cattle naturally infected by *Babesia*. Spp

INTRODUCTION

Babesiosis is an emerging, tick-transmitted, zoonotic disease caused by haematotropic parasites of the genus *Babesia*, *Babesia* parasites are some of the most widespread blood parasite in the world, and consequently have considerable world wide economic, medical and veterinary impact. The parasite is intraerythrocytic and is commonly called piroplasmosis due to pear-shaped forms found within the infected red blood cells.

Babesia bovis, a hemoprotozoan parasite invading and replicating within bovine erythrocytes, causes serious disease manifestations characterized by fever, anemia, hypertensive shock syndrome and in sever cases a fetal cerebral disease with a high mortality rate (Wright *et al.*, 1988 and Homer *et al.*, 2000). Therefore the spread of infection result in great economic losses all over the world (Brown and Palmer, 1999).

Recently sialic acid (SAs), the terminal or sub terminal non-reducing units attached to the underlying galactose residue of many sialoglycoprotein on the host cell membrane (Varki, 2001) have been shown to play an important role in RBCs invasion by *Babesia* parasites (Kania *et al.*, 1995 and Zintl *et al.*, 2002), the (SAs) residues' regardless of the type of linkages (α 2-3 and α 2-6 linkages) are important in RBCs invasion by *Babesia.bovis* which can invade both bovin RBCs, human and other animal RBCs in an SA-dependent manner. Gaffar *et al.* (2003).

Adenosine deaminase (ADA) is an enzyme that catalyses hydrolytic deamination of either adenosine or deoxyadenosine to produce inosine and deoxyinosine respectively (Ungere *et al.*, 1992). In domestic animals. ADA is present in all organs, although the highest activity has been found in lymphoid tissues in animals. ADA and its isoenzyme activities in the spleen, lymph nodes and thymus have been found in high levels and in the brain, adrenal

gland, muscles, kidney and liver in low level (Tanabe, 1993). It has been detected in the cell cytoplasm and nucleus (Zuck and Rotzsch, 1990). Furthermore, its enzymatic activity has also been reported in blood cells, serum and plasma (Katopodis and Stock, 1980).

The main biological activity of ADA is to protect lymphocytes from toxic effects of 2-deoxyadenosine, deoxyadenosine triphosphate and deoxyadenosine diphosphate. Which depress immune function. It has been shown that ADA is related to normal condition involving lymphocyte-monocyte proliferation (Kaplan *et al.*, 1985; Baganha *et al.*, 1990).

The aim of the present study was to evaluated the cattle immune response by determine the serum total sialic acid and adenosine deaminase levels in cattle naturally infected with babesiosis.

MATERIALS and METHODS

1- Animals

A total number of 50 female's cattle, 5-7 years old, from private farm in Assuit Governorate were used as subjects for this study. The cattle were divided into two groups according to the clinical and parasitological examinations. 40 female cattle suffering from Babesiosis and used as a diseased group (1). The rest, 10 females were clinical and laboratory healthy cattle and used as control group (2).

2- Clinical examination:

All the selected animals' cattle were subjected to clinical examination. The infected animals showed clinical signs as a high rectal temperature reaching 41.5 °C, anorexia, the mucous membranes' are first injected and reddened, but as erythrocytic lyses occurs, the color changes to the pallor of anemia. This is usually associated with sever hemoglobinuria and hemoglobinemia. Hemolytic anemia and jaundice were evident

in advanced stage of infection and beside that all the infected animal are infested with ticks.

3- Samples:

1- Blood samples:

a) Whole blood samples:

Blood samples were taken into EDTA containing vacutainer tubes from the jugular vein for blood film preparation for parasitological examination (*Babesia. Spp*).

b) Blood samples without anticoagulant:

Serum samples were separated by centrifugation at 3000 rpm at room temperature and stored at -20°C until biochemical analysis.

2- Fecal samples:

Ten grams of fecal samples were collected from each animal in plastic pages and screened for parasitic infection by concentration flotation technique according to (Soulsby, 1986).

3- Parasitological examination:

EDTA-blood was used to prepare thin and thick blood film from each animal, then fixed in methanol, stained with Giemsa stain and examined by oil immersion lense under microscope. The degree of parasitemia was recorded as the percentage of the infected red blood per 100 cells counted (0.5%, 1%, etc) as in Table (1), according to Saunders (2007). The smear was recorded as negative if no piroplasma bodies were observed in 200 fields/ animal.

Ten grams of fecal sample were screened macroscopically and microscopically for parasitic infestation using standard methods.

4- Biochemical analysis:

Total sialic acid concentration was determined according to Sydow *et al.* (1988). Briefly, 400 µl of serum were treated with 3ml of 5% perchloric acid for 5minutes at 100 °C and centrifuged at 1800 rpm.

Two ml of supernatant were mixed with 400/ul of Ehrlich reagent (5g dimethylaminobenzaldehyde /50 ml HCL with 50 ml of distilled water). Incubation for 15 minute at 100c was made. Finally the optical density was read with spectrophotometer (UV-

1201.Shimadzu, Japan). While lipid bound sialic acid concentration was determinate according to Katopodis *et al.* (1980) and protein bound sialic acid were calculate by subtracted lipid bound sialic acid from total sialic acid.

Serum (ADA) activity was determined spectrophotometrically according to. Giusti and Galanti, (1984), where colorimetric method based on the principle of measuring absorbance of color at 628 nm.

5- Statistical analysis.

The result was analyzed by on-way ANOVA followed by Duncan test using computer software. Differences were considered statistically significant when $P < 0.05$ against control group. All the values were presented as mean \pm SEM.

RESULTS

Clinical examination:

The most clinical signs of infected cattle with *Babesia* were hemoglobinuria, increase of rectal temperature (40 °c-41.5° C). Animals commonly develop incoordination and depression and go down with the head extended but later thrown back with involuntary movement of legs. These signs are followed by death.

Parasitological examination:

A- Blood films prepared from diseased cattle indicated the presence of *Babesia .Spp* in the red blood cells with different degree of parasitism (0.5%- 2%) as in Table 1. While examination of blood films from control group indicated no piroplasma.

B- Fecal examination (Gross and microscopically) in all animals included in these study indicated that all animals were free from internal parasites to avoid the cross reaction which may be occur with the results of babesiosis.

Biochemical analysis:

Mean serum sialic acid concentration of these study were summarized in Table 2, which indicated marked significantly ($p > 0.05$) increase in the concentration of sialic acid in the diseased cattle than in the

control. However the individual rates of parasitemia were not correlated with sialic acid concentration ($r=0.027$, $p>0.05$). Mean value of serum (ADA) activity are presented in Table 2, where significant

increase ($p> 0.05$) in (ADA) levels were detected in diseased group than the control group.

Table 1: Degree of parasitism in the animals of the present study.

Parasitemia	Parasite/ ul of blood	Clinical correlation
0.5%	5-20	Number of organism that required for positive thick film (sensitivity) in (4animals)
1%	20-80	The animal may show of clinical signs in (8animals)
1.5%	80-320	The animals with moderate clinical signs in (20 animals)
2%	320-1000	Sever clinical signs of animals with increase mortality in (8 animals)

Table 2: Statistical analysis of serum total sialic acid and adenosine deaminase enzyme levels in cattle naturally infected with *Babesia. Spp* and healthy control group.

Animals Parameters	Control group (n= 10)	Diseased group (n=40)
TSA mg/dl	57.6±22.88	102.09±3.93*
ADA u/l	11.07±44.35	65.18±16.30*

DISCUSSION

The present study was conducted to examine the effect of *Babesia* infection on serum sialic acid concentration and adenosine deaminase enzyme activity in cattle.

Our finding indicated that *Babesia* infection induced significant ($p>0.05$) increase in total sialic acid and adenosine deaminase activity.

It is currently unknown how babesia infection lead to the increase in sialic acid content. However, Eslevo *et al.* (1982) and Ertekin *et al.* (2000) reported significant increase of serum sialic acid in cattle infected with *Thieleria* or *anaplasma*. In addition Olaniyi *et al.* (2001) reported an elevation in serum sialic acid concentration in trypanosomes. Conversely sialic acid deficiency in the erythrocytes of infected

cattle with *babesia bovis* has been reported by Gaffar *et al.* (2003).

The increase in sialic acid may be attributed to the fact that sialic acid could modulate biological cell-cell interaction in two non – mutually exclusive ways, sialic acid could mask the underlying sugar chain (i.e. lactosaminic sequema), hindering them from interacting with galactose-specific lectins (Galectins) as recorded by Razi and Varki (1998). Second way, sialic acid would directly interact with specific sialic acid binding lectins (Sigles). Therefore increase content of sialic acid would interfere with attachment of sporozoites on host cells or promote the invasion of erythrocytes by merozoites (Dallolio, 2000).

Host sialic acid plays an important role in erythrocytes invasion by babesia parasite.

Afact that agree with Yokpyama *et al.* (2006), where sialic acid play as a host receptor in the erythrocytes invasion by *Babesia bovis*. Treatment prevented the increase of sialic acid (Gaffar *et al.*, 2003). In addition, Okamura *et al.* (2005), observed that sialic acid residues on host RBCs play an important role in the erythrocyte infection by *Babesia Caballi* and *Babesia equi* and cause significant increase in its concentration.

Adenosine deaminase enzyme level is increased with many diseases due to the stimulation of cellular immunity. In the present study serum ADA was significantly increased ($p<0.05$) in the diseased group in contrast to the control group. The increase in serum (ADA) came in agreement with Kontas and Salmonglu (2006).

The increase of (ADA) may be attributed to erythrocytic damage caused by the parasite and/or the phagocytic activity of macrophages (Ustundage *et al.*, 1999).

Finally we can be conclude that *Babesia* infection in cattle induced marked increase of serum sialic acid concentration and adenosine deaminase enzyme level, suggesting that these indicators would indirectly promote the invasion of the parasite in host.

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