

EVALUATION OF SERUM SIALIC ACID AND ADENOSINE DEAMINASE ACTIVITY AS A DIAGNOSTIC INDICATORS FOR BABESIOSIS (B.OVIS) WITH SOME CLINICAL AND BIOCHEMICAL STUDIES IN SHEEP NATURALLY INFECTED WITH BABESIA IN ISMAILIA GOVERNORATE

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ABSTRACT

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The study was conducted on one hundred and ten adult sheep from private farms in different localities in Ismailia Governorate. Which Ninety sheep were naturally infected with Babesia ovis and Twenty sheep were proved by clinical and laboratory method to be healthy and used as control. The clinical signs of diseased sheep revealed a marked hyperthermia, loss of appetite, depression, weakness, cessation of rumination, extreme pale mucus membrane due to severe anaemia, increased respiratory rate and labored breathing. The urine was dark red to brown coloration and later on jaundice subsequently developed. The biochemical data revealed that a highly significant increase of both serum sialic acid and adenosine deaminase activity in infected sheep in comparison with healthy one. In addition total bilirubin, direct and indirect levels were highly significant increase in infected sheep when compared with the healthy one. Total protein was significantly decreased with hypo-albuminaemia, while globulin, glucose and cholesterol were non-significantly increased. Blood serum calcium and sodium levels showed a significant decreased, while serum level of inorganic phosphorus was significantly increased in infected animals. Blood serum iron revealed highly significant elevation, while copper and zinc levels showed highly significant decreased. Also significant increase in AST, ALT and AP were evident in infected sheep comparatively with control.

تقييم حمض السيلاليك ونشاط انزيم الادينوسين دى امينيز ككواشف تشخيصية للبابيزيا مع دراسات اكلينيكية وبيوكيميائية فى مصل دم الاغنام المصابة طبيعيا بالبابيزيا بمحافظة الاسماعيلية

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يعد مرض حمى البول الدموى (البابيزيا) عند الاغنام احد الامراض الناجمة عن الاصابة بالاوليات ويسبب هذا المرض طفيليات بذرية وحيدة الخلية صغيرة الحجم تنتمى الى جنس البابيزيا. وقد اجريت الدراسة على عدد 90 من الاغنام المصابة طبيعيا بالبابيزيا بالاضافة الى عدد 20 من الاغنام السليمة واستخدمت كضابط للتجربة. تتميز اعراض هذا المرض بارتفاع شديد فى درجة حرارة جسم الحيوان مع فقدان للشهية وتوقف الاجترار وهزال وضعف مع هبوط عام وزيادة عدد دقات القلب وصعوبة التنفس مع ظهور برقان على الاغشية المخاطية وتلون البول باللون الاحمر الداكن. وقد اظهرت نتائج التحاليل البيوكيميائية لمصل دم الاغنام المصابة الى زيادة معنوية فى مستوى كلاً من حمض السيلاليك ونشاط انزيم الادينوسين دى امينيز والبيوروبين الكلى والمباشر والغير مباشر ونقص معنوى فى البروتين الكلى والاليومين بالاضافة الى وجود انخفاض معنوى لكل من الكالسيوم والصوديوم مع زيادة فى الفوسفور كما وجدت زيادة معنوية عالية فى عنصر الحديد بينما انخفض مستوى كلاً من النحاس والزنك كما اظهرت الدراسة ايضا وجود زيادة

معنوية في مستوى كلا من انزيم الاسبرتات امينو ترانسفيريز والالكلين فوسفاتيز. ان اصابة الاغنام بحمى البول الدموي يسبب خسائر اقتصادية كبيرة وهذا يتطلب بذل جهود كبيرة للتحكم ومن ثم التخلص من هذا المرض. لذا ينصح باجراء مكافحه دورية للقراد بالمبيدات الحشرية غير الضارة بصحة الحيوان والبيئة لان القراد يقوم بدور الناقل لمرض حمى البول الدموي.

Key words: *Babesia, sheep, sialic acid, adenosine deaminase activity.*

INTRODUCTION

Diseases of small ruminants are among the least recognized problems in veterinary science, this is true especially for tick-borne diseases of sheep (Friedhoff, 1997). *Babesia* is one of the most important diseases in the tropical and subtropical countries. The severity of the disease increases with the long exposure to stress factors, which lower the productive performance of the animals. Ramadan and Lubna, (2000), Radwan and Ali, (2004) and Sulaiman *et al.* (2010).

Babesia species are intraerythrocytic protozoan of domestic and wild animals caused anaemia and hemoglobinuria. Among the *Babesia* species affecting small ruminants and incriminated for major economic losses, such loss result from deaths of affected sheep, unthriftiness of chronic cases and cause the greatest economic losses in sheep and goat production (Radwan and Ali, (2004); Sulaiman *et al.* (2010).

Sheep Babesiosis caused by *Babesia ovis* and *Babesia motasi* in the area infested with *Rhipicephalus bursa*, morbidity and mortality due to Babesiosis in sheep corresponded closely to the seasonal activity of vector *Rhipicephalus bursa*, the peak was in summer followed by spring (Ramadan and Lubna, 2000; Yasin, 2003).

Sialic acid (SA) an acetylated derivative of neuraminic acid, is widely distributed in mammal tissues and body fluids.

The majority of sialic acids are found in either protein or lipid bounded forms, while little amount is in the free forms (Col and Uslu, 2007). Therefore, the detection of sialic acids be a valuable indicator for diagnosis and prognosis of inflammatory diseases (Motoi *et al.*, 1984).

While Adenosine Deaminase (ADA) is an enzyme that is present in all cells. ADA activity is elevated in many diseases where cellular immunity is stimulated (Ustundag *et al.*, 1999). Measurement of ADA is used for the diagnosis and monitoring of autoimmune and inflammatory diseases because of its easy identification, high sensitivity and low cost (Maldhure *et al.*, 1994).

The aim of the present study was oriented to evaluate the host immune response by the use of serum total sialic acid and ADA activity in sheep naturally affected with babesiosis and investigate the effect of naturally infected sheep with *Babesia* upon observed clinical signs and some serum biochemical parameters.

MATERIALS and METHODS

I- Animals: One hundred and ten adult sheep of both sex, 2-5 years old and 35 - 55 Kg body weight from a private farms in different localities in Ismailia Governorate. Where as Ninety sheep were naturally infected with *Babesia ovis*, in addition to Twenty sheep were clinically healthy used as control. All animals were subjected to careful clinical and laboratory investigation to insure their healthy status according to Pugh, (2002).

II- Samples:

A- Blood smears: Thin blood smears were collected from the ear vein of all the examined animals and individually prepared. Fixed blood films were stained with Giemsa stain for identification of blood parasite that carried out in parasitology department, Faculty of Veterinary Medicine, Suez Canal University, according to Meyer *et al.* (1992).

B- Blood samples: were collected from the jugular vein in dry sterilized clean centrifuge tubes from each animal and allowed to clot at room temperature, then centrifuged at 3000

r.p.m for 20 minutes to separate clear non-hemolysed serum. Firstly, serum samples were colorimetrically analysed using test kits (Bicon–Germany) for measuring glucose levels as soon as possible. While the collected clear sera were kept at -20°C till used for biochemical assay. Serum Sialic acid was measured according to the method reported previously by Sydow *et al.* (1988) while Adenosine deaminase activity (ADA) was determined by the use of the Giusti method (Giusti and Galanti, 1984) a colourimetric method based on the principle of measuring absorbance of the coloured indophenole complex at 628 nm.

Blood serum cholesterol, total bilirubin, direct bilirubin, total protein, Albumin, AST, ALT, ALP, Calcium, Phosphorus and Magnesium were determined spectrophotometrically used standardised test-kits supplied from Bio- Merieux (Bains/France).

Serum copper, Iron and zinc levels were determined using atomic absorption spectrophotometrically (Perkin Elmer Model 2380 USA) In Ismailia Agriculture Research Institute, Soil and Irrigation Department.

Blood serum indirect bilirubin, globulin and albumin/ globulin ratio were calculated mathematically.

III- Statistical analysis: The obtained data was subjected to software program according to Selvin, (1996) to study the effect of Babesiosis on some serum biochemical parameters.

RESULTS

Clinically infected sheep showed different signs graduated from marked increase of temperature, loss of appetite, depression, weakness, emaciation, extreme pale mucous membrane, increased respiratory rate, labored breathing, haemoglobinuria, where the urine was dark red brown color and jaundice subsequently developed.

As well as presence of ticks (*Rhipicephalus* spp.) were detected on different parts of the body. Examination of blood films of diseased sheep revealed infection with *Babesia ovis*.

The results of biochemical analysis of blood sera of infected and controlled sheep are illustrated in Tables (1, 2, 3).

Table 1: Effect of *Babesia ovis* infection on some serum biochemical parameters in sheep.

| Parameters | Clinically healthy sheep n=20 | Infected sheep with Babesia n=90 |
|------------------------------|----------------------------------|-------------------------------------|
| Sialic acid(mg/dl) | 59.82 ± 2.34 | 75.60±2.75 *** |
| Adenosine deaminase (U/L) | 7.64± 0.3 | 16.52±2.60 *** |
| Glucose(mg/dl) | 52.20± 3.6 | 59.8±2.9 |
| Cholesterol(mg/dl) | 135.92±4.5 | 142.7±5.2 |
| Total bilirubin(mg/dl) | 0.43±0.05 | 1.45±0.23 ** |
| Direct bilirubin(mg/dl) | 0.14±0.02 | 0.28±0.06 ** |
| Indirect bilirubin(mg/dl) | 0.29±0.03 | 1.17±0.17 ** |
| Total protein(gm/dl) | 7.45±0.05 | 6.44±0.07 * |
| Albumin(gm/dl) | 3.26±0.02 | 1.96±0.03 * |
| Globulin(gm/dl) | 4.19±0.03 | 4.48±0.04 |
| A/G ratio % | 0.78±0.67 | 0.44±0.75 * |

The obtained results were mean ± SE.

* Significant at (P<0.05).

** Highly Significant at (P<0.01)

*** Very highly significant at (p<0.001)

Table 2: Effect of *Babesia ovis* infection on some blood serum biochemical macro and micro

elements in sheep.

| Parameters | Clinically healthy sheep n=20 | Infected sheep with Babesia n=90 |
|------------------------------|----------------------------------|-------------------------------------|
| Calcium (mg/dl) | 10.21±0.23 | 7.2±0.35 * |
| Inorganic phosphorus (mg/dl) | 6.85±0.90 | 8.45±0.50 * |
| Magnesium (mg/dl) | 2.56±0.25 | 2.71±0.16 |
| Sodium (mmol/L) | 147.24±2.30 | 130.80±2.10 ** |
| Potassium (mmol/L) | 5.72±0.15 | 5.86±0.17 |
| Iron (µg/dl) | 148.60±6.84 | 253.70±8.11 *** |
| Copper (µg/dl) | 94.10±4.27 | 67.52±4.30 ** |
| Zinc (µg/dl) | 95.42±4.30 | 71.75±3.20 ** |

The obtained results were mean ± SE.

* Significant at (P< 0.05)

** Highly Significant at (p< 0.01)

*** Very highly Significant at (p<0.001)

Table 3: Effect of Babesia ovis infection on some blood serum enzymes in sheep.

| Parameters | Clinically healthy sheep n=20 | Infected sheep with Babesia n=90 |
|---------------|----------------------------------|-------------------------------------|
| A S T (I.U/L) | 48.75±2.8 | 63.92±4.13 ** |
| A L T (I.U/L) | 26.3±1.92 | 47.26±3.84 ** |
| ALP (I.U/L) | 119.64±4.30 | 145.52±3.22 *** |

The obtained results were mean ± SE.

** Significant at (P<0.01)

*** Very highly significant at (p<0.001)

DISCUSSION

Babesia is a small protozoan found in vertebrates red blood cells. This protozoan can be found in many species of animals such as cow, sheep, goat, pig, horse and buffalo. Babesiosis is an acute hemolytic febrile disease which is more prevalent in domesticated animals and transmitted by hard tick's bite, prevalence of this disease in tropical and subtropical regions is more and it causes severe loss of sensitive animals, (Seyyed *et al.*, 2011).

Mortality of this disease in absence of treatment in case of Babesia ovis infection may reach 70% - 80% in sheep, (Hosseini, 2003).

The difference of severity of the disease may attribute to the higher incidence of tick vectors and the change in husbandry, climatic

condition and control of ticks with respect to the seasonal variation that the peak was in summer followed by spring that agreed with Yeruham *et al.* (1998), Ramadan and Lubna, (2000).

Clinical examination among diseased sheep showed that the affected sheep had marked increase of temperature, loss of appetite, emaciation, pale mucus membranes, jaundice dry muzzle and cessation of rumination, increased respiratory rate, muscle tremors in addition to the urine was dark red to brown in color due to hemoglobinuria in the final stages. Similar signs were recorded by El-Sawahly (1999); Omran and Abd El-Azeim (2000); Radwan and Ali (2004); Sulaiman *et al.* (2010).

Serum biochemical changes in sheep infected with Babesia ovis has been documented. The results revealed that increased level of serum

sialic acid concentration in sheep infected with *Babesia ovis* in comparison with the control group.

The results of the present study are similar to the previous reports by Ertekin *et al.* (2000); Karagenc *et al.* (2005). The elevation of serum sialic acid concentrations in sheep with Babesiosis may represent the host immune response to the parasite, where the increased level of sialic acid may alter receptor-ligand interactions, which are known to play an important role in inflammation and immune response (Karagenc *et al.*, 2005).

In addition the release of sialic acid from the glycolipids or glycoproteins of the lysed cell membrane surfaces may result in the elevation of serum total sialic acid level in infected sheep.

Serum Adenosine deaminase levels was significantly higher ($P < 0.001$) in the infected groups in contrast to the control group. This increase in serum ADA levels may result from the phagocytic activity of macrophages and / or erythrocyte damage caused by the parasite (Ustundag *et al.*, 1999).

Babesia ovis infection induced marked and persistent elevations of serum total sialic acid concentration and serum ADA suggesting that these indicators would indirectly promote the invasion and presence of the parasite in the host. These results were in agreement with Deger (2007).

Non-significant increase in serum glucose and cholesterol were observed in infected sheep in comparison to healthy animals. These findings indicating liver disease and nephrotoxic syndrome. Similar results were previously obtained by Abou EL-Naga (2002); Radwan and Ali (2004).

The results indicated also that, highly significant increase in total bilirubin (direct and indirect) in infected sheep comparatively with healthy animals. These results emphasize liver damage and increased in the indirect bilirubin was due to erythrocyte haemolysis (Jain, 2000). Similar results were reported by Yeruham *et al.* (1998); Abou El-

Naga, (2002); Sulaiman *et al.* (2010).

Total protein and albumin were significantly decreased, these results may be due to destructive effect of *Babesia* on liver cells producing liver damage resulting in impaired synthesis of total protein and albumin from the liver (El-Sawahly, 1999) or due to renal insufficiency caused by the nephrotoxic effect when hemoglobin released from the ruptured red blood corpuscles due to parasite multiplication. These results agreed with those reported by Abou El-Naga (2002); Sulaiman *et al.* (2010). While non-significant increase in serum globulin level in infected sheep was attributed to immune response of animal body to the infection Omran and Abd El-Azeim (2000); Rdwan and Ali (2004).

The effect of *Babesia* on serum calcium and sodium levels showed a significant decrease while serum level of inorganic phosphorus was increased in infected animal in comparison to healthy one.

The obtained results may be due to renal insufficiency caused by the nephrotoxic effect of hemoglobin that results from the rupture of red blood corpuscles due to parasite multiplication, also by liver involvement and anorexia for hypocalcaemia as well as the haemolytic nature of anaemia.

The obtained results coincided with those previously observed by Habela *et al.* (1991); Abou El-Naga (2002). On the other hand, no significant difference in serum potassium and magnesium levels were observed. Such results agree with Abou El-Naga (2002).

Blood serum iron level revealed highly significant elevation, these results were similar reported by El-Saifi *et al.* (1990); Omran and Abd El-Azeim (2000). Who attributed this alterations to the intra-vascular hemolysis. Blood serum copper and zinc showed highly significant decrease which may attributed to liver and bone marrow involvement as well as copper depletion (Ceci *et al.*, 1997).

A highly significant increase in Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were observed in infected

sheep in comparison to healthy one.

Such elevation was due to cellular damage caused by Babesia organisms lead to intravascular hemolysis which lead to anoxia and inflammatory lesions in various organs especially liver and kidneys causing nephrotoxic effects and lysis of erythrocytes during Babesia infection, these results were agreed with the results obtained by Radwan and Ali (2004); Sulaiman *et al.* (2010) in similar condition.

Finally, it may concluded that, the increased levels of serum sialic acid and Adenosine deaminase activity may mimic induced host immune response. Where Babesia ovis caused several pathological effects in many organs and tissues specially liver and kidneys to the animal host which lead to biochemical, metabolic and electrolytes disorders in blood serum of the affected animals.

The increased levels of serum sialic acid and adenosine deaminase activity may be used as a diagnostic indicators in diagnosis of Babesiosis in sheep, but these elevations are not definite diagnosis for Babesiosis because it may be increased also in many other disease conditions.

So, it must be kept in mind that while treatment of diseased animals by eradication of ticks associated with additional treatment containing mineral mixture and injection of electrolytes solutions.

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