COMPARATIVE STUDY OF COAGULATION PROFILE, OSMOTIC FRAGILITY AND CATION CONCENTRATION BETWEEN NORMAL AND *THEILERIA* INFECTED CATTLE

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ABSTRACT

Coagulation profile revealed that the activated partial thromboplastin time (APTT) and platelets count of *theileria* infected bulls were decreased significantly than the normal ones. The plasma and intra-erythrocytic levels of Na, k, Ca, Zn, Cu, and Fe did not change significantly during bovine theileriosis. Hemoglobin and packed cell volume were markedly decreased in the infected group. The erythrocytic osmotic fragility in infected bulls was about 3, 4 and 3 times greater than those observed in normal group at 0.8, 0.7 and 0.6% NaCl, respectively.

Keywords: Bovine theileriosis, The erythrocytic osmotic fragility, Hemoglobin, Packed cell volume, Coagulation profile, Intra and extra-erythrocytic concentrations of bulk (Na, K, and Ca) and Trace cations (Zn, Cu and Fe).

INTRODUCTION

Tropical theileriosis is a tick- borne protozoal disease of cattle and buffalo caused by *Theileria annulata* and characterized by fever, enlargement of superficial lymph nodes, wasting progressive anemia and jaundice. The disease was found in the Mediterranean and subtropical regions of the world (**Radostits *et al.*, 1994**). Tropical theileriosis is a major constraint to livestock improvement programs in many parts of the middle East and Asia where 200 million cattle were said to be at risk of infection (**Brown, 1990**).

Theileriosis is one of the most devastating blood parasite-affecting cattle in Saudi Arabia. The parasite existence was reported in various provinces as Gizan, Hofof and Al-Qassim regions (**Magzoub *et al.*, 1992 and El-Metenawy, 1996 and 2000**).

The red blood cells are destroyed by the host reticuloendothelial system, presumably by phagocytosis. Structural and physiological changes

taking place at the red cell level contribute to the increased erythrocyte sensitivity to phagocytes and / or to make the cells susceptible to autoimmune attack (**Schroeder and Ristic, 1968**). Disturbance in energy supplying mechanisms may be a pathway for ultimate red blood cell destruction, producing alterations, particularly those related to the active movement of cations across the cell membrane (**Valentina, 1975**). Permeability impairment of such cells could be partially responsible for the pathology of the disease. The maintenance of the ionic symmetry between the plasma and the cytoplasm depends on the activity of the membrane ATPases (**Brewer, 1974**). However, the previously mentioned investigations were, surpassingly, lacking information about the coagulation profiles and intra-erythrocytic cation levels.

Therefore, the present study aimed to investigate the effect of *Theileria annulata* on coagulation profile, erythrocytic osmotic fragility (E.O.S.F) as well as intra and extra-erythrocytic concentrations of bulk (Na, K, and Ca) and trace cations (Zn, Cu and Fe).

MATERIAL AND METHODS

1-Animals:

This study was conducted on 27 Holstein-Friessian bulls. The age of the animals was ranged from 1-2 years and were housed in the same experimental facilities belonged to private farm at Al-Qassim area (Saudi Arabia). The animals were divided into two groups; the diseased (17) bulls, and control (10) animals. The diseased bulls showed fever, depression, reduced appetite, weakness, labored breathing, and enlargement of superficial lymph nodes and oculo-nasal discharge. The control group was clinically healthy, proved to be negative for blood parasites through 3 successive Giemsa stained blood films. All individuals of both groups were found free from gastrointestinal parasites as revealed by **Soulsby, (1982)**.

All animals were fed twice a day with commercial concentrates according to **INRA, (1978)**. Calcium (ground limestone) and salt (Electral Aesculaop, Belgium) were added in order to avoid Ca/P and electrolyte imbalance. Water was available ad libitum.

2- Blood sampling:

Triplicate blood samples were obtained by jugular venepuncture. The first sample were drawn into polyethylene tubes containing 0.13M trisodium citrate (9:1- v/v) and placed on an ice bath at 2 to 4 °C for coagulation assays. The second sample was collected into tubes containing EDTA for haematological examination while the third tubes were containing lithium heparin for erythrocyte osmotic fragility and cation concentrations.

3- Laboratory procedure:

Collection and preservation of blood samples were done according to **Benjamin, (1984)**.

A- Parasitological examination:

Parasitisemia was determined by examination of both thin and thick blood smears that stained with Giemsa according to **Levine, (1985)**. The stained blood films were carefully examined until positive or 100 fields had been examined. For estimation of parasitisemia in a positive sample, only areas of the thin film where the RBCs are 1 cell thick and not overlapping but touching should be examined. The number of RBCs in one of these fields should be counted and estimation made on 10 fields. The number of parasitized cells should be counted in 10 fields and an average was taken. The degree of parasitaemia was calculated from the following equation **(Fleck and Moody, 1993)**.

$$\% \text{ Parasitaemia} = \frac{\text{average no. parasitized r.b.c.s per field x100}}{\text{average no. of RBCs per field}}$$

Ticks were carefully collected from the infested bulls and preserved in 70% ethanol for the proper identification after **Hoogstraal, (1956)** and **Hoogstraal *et al.*, (1981)**.

B- Coagulation assays:

Citrated blood was processed within an hour of collection and centrifuged for 20 min (100g at 4°C). Plasma was separated into plastic capped vials and coagulation assay was carried out within 2-3 hours from collection. Coagulation assays of extrinsic (prothrombin time- PT and factor V11) and intrinsic (activated partial prothrombin time- APPT and factor V111) pathways were measured using a commercial kits (BioMerieux-France). The coagulation assay was performed in duplicates and the average was recorded.

C- Haematological examination:

Blood platelets count using Haemocytometer, Packed cell volume (PCV) determination using microhaematocrite method as well as photometric measured using Drabbkin solution were done for Hemoglobin (Hb) estimation according to **Coles, (1986)**. Moreover, mean corpuscular hemoglobin contents (MCHC) were calculated using the obtained PCV and Hb **(Benjamin, 1984)**. Fragility test was recorded after centrifugation of heparinized blood (plasma were separated for electrolyte determination). The precipitated RBCs were washed 3 times with normal saline (0.85%). Cells were ready for fragility test **(Abdel-Rahman *et al.*, 1994 and Abdel-Rahman and El-Mougy, 1995)**.

D- Cations analysis:

Sodium and potassium concentrations were assayed by emission flame photometry, whereas Ca, Zn, Cu, and Fe were determined using atomic absorption spectophotometry as described by **Clegg *et al.*, (1981)**.

The intra-erythrocytic (E) cation concentrations were recorded in hemolysed blood by diluting 0.35 ml of lithium heparin -blood samples with 1.0 ml of deionized water. The previous mentioned cations were then calculated according to **Mulei and Daniel, (1988)**. Heparinized blood samples were centrifuged for 15min (4500g). The supernatant plasma (p) was siphoned off into plastic capped vials and stored at -20°C pending cations analysis.

RESULTS

The collected ticks from the infested bulls were identified as *Hyalomma anatolicum;* where the blood parasite was identified as *Theileria annulata* according to the symptoms of the disease, morphological characters of the parasite (both piroplasm and Koch 's blue bodies) and the recognization of the vector. The percent of parasitisemia among the infected bulls ranged from 0.01 to 5 % with an average of 1.59 %.

A significant decrease was recorded in APTT ($p<0.05$) and thrombocytopenia ($p<0.01$) in *Theileria*-infested bulls in comparison with control ones (Table 1). Other coagulation tests (PT, V11and V111) showed in significant change between the two groups.

Erythrocytes of *Theileria*-infected animals showed a higher osmotic fragility ($p<0.01$) in relative to cells of control group (Fig. 1).

The hemolytic effect observed in *Theileria* infected red cells was even at 0.9% NaCl (1.32%) and 0.8% NaCl (2.76%). The hemolytic effect was significantly ($p<0.01$) increased 4 times (6.8%) and 3 times (35%) when compared with control red cells (1.7&11.7%) at 0.7 and 0.6% NaCl, respectively.

Hematological examination of *Theileria*- infected animals revealed that the hemoglobin concentration ($p<0.01$) and packed cell volume ($p<0.05$) were markedly decreased (Table 2). Table (3) shows the bulk (Na, K and Ca) and trace (Zn, Cu and Fe) cation concentrations in extra (plasma) and intra-erythrocytes for both control and *theileria*- infected bulls. No significant change seems to occur in the cationic balance of the erythrocytes as a consequence of the infection. Generally, the levels of intra-erythrocytic K, Ca, Zn, Cu and Fe were higher than plasma levels by 10, 6, 15, 6 and 20 times, respectively.

DISCUSSION

The intrinsic pathway of coagulation is initiated when blood contacts an incompatible surface (rough, non-wettable or negatively charged) (**Reece, 1992**). Any surface other than the intact endothelial lining of the blood vessel wall is considered foreign to both plasma coagulation factors and the blood cells to initiate the intrinsic coagulation pathway (**Ruckebusch *et al.*, 1991**).

The recorded shortening of the APTT in the present study may be seen in conjunction with an activated coagulation system for instance in a compensated, low-grade consumptive coagulopathy (**Henry, 1984**).

Disseminated intravascular coagulopathy (DIC) is characterized by an augmentation of normal clotting mechanisms which results in depletion of coagulation factors (V, V11, V111 and platelets) and deposition of fibrin clots in microvasculature. This fibrin clots decrease tissue perfusion which may lead to further activation and depletion of clotting factors by the release of tissue thromboplastin as a result of tissue hypoxia (**Radostits *et al.*, 1994**).

The occurrence of DIC could be suspected, where there is a combination of the following red blood cells distortion and fragmentation produced by damage of RBCs during passage through microvascular thrombi (**Moore, 1979**). In the same manner, DIC could be suspected in *theileria*-infected red cells due to significant fragility recorded in the present study.

Theileria-infected animals showed a significant ($P<0.01$) thrombocytopenia ($189x10^3$ /mi). The decreased number of circulating platelets could be resulted from a wide variety of causes as intravascular hemolysis and increased consumption or destruction (**Burstein and Harker, 1981**). The appearance of haemorrhage was directly related to the number of circulating platelets and bleeding was seen when platelet number fell below 5000/ml (**Radostits *et al.*, 1994**). In the present study, the recorded thrombocytopenia did not reach to the previous level of bleeding, so haemorrhage was not recorded in *theileria*- infected bulls.

The in vitro osmotic behavior of the erythrocytes from *theileria*-infected animals became more susceptible to lysis in NaCl (Fig. 1). The concentrations of measured intra-erythrocytic cations did not change during the development of the disease (Table 3). The alteration in the osmotic response to NaCl could not be related to changes in these cations. The intracellular presence of the parasite may contribute with osmotically active solutes and / or alter the integrity of cell membrane, thus enhancing the entrance of other solutes as explained by **Silva *et al.*, (1989)** in *Anaplasma*.

Fever is one of the clinical singes of theileriosis among the infected animals, which may affect cell membrane properties. The erythrocytic membrane is lipids and protein in nature, which could be altered with effect of temperature on the E.O.S.F (**Aloni *et al.*, 1977 and Abdel-Rahman *et al.*, 1994**). The increase of E.O.S.F. in the present study may be due to changes in ultrastructure and membrane protein composition as occur in bovine

anaplasmosis (**Nordelo and Ysern-Caldentey, 1982 and Giardina *et al.*, 1983**).

Hematological studies of *theileria-* infected group revealed a significant progressive decrease in hemoglobin concentration and packed cell volume. The result agrees with the studies of **Sandhu *et al.*, (1998)**.

In the present study, there is no significant difference in the concentrations of the intra and extra- erythrocytic cations (Na, K, Ca, Zn, Cu and Fe). This may be due to an increase activity of the ionic-dependent ATPase, which could maintain the cellular levels of these cations as occurs in bovine anaplasmosis (**Silva *et al.*, 1989**).

The observed low ENa and high EK concentration in comparison to PNa and PK agrees with the studies in sheep and goat (**0**), man and horse (**Agar and Board, 1983**), Cattle (**Mulei and Daniel, 1988**) and camel (**Ibrahim *et al.*, 1994**). On the other hand, red cells of carnivorous (dog and cat) had high intra- cellular Na and low K levels (**Agar and Board, 1983**) and some types of bovine breeds of low-K type (**Christinaz and Schatzman, 1972**).

REFERENCES

Abdel-Rahman, H.A.; El-Kascahab, S.H. and El-Mougy, S.A. (1994): A comparative study on the effect of temperature and pH on the erythrocytes osmotic fragility of some sheep breeds in Saudi Arabia. World Rev. Anim. Prod. 29 (1): 34-39.

Abdel-Rahman, H.A.and El-Mougy, S.A. (1995): The use of acetylsalicylic acid as preservative to prevent erythrocyte osmotic fragility. World Rev. Anim. Prod. 30 (1&2): 50-53.

Agar, N. S. and Board, P.G. (1983): Red blood cells of domestic mammals. Elsevier Amsterdam. New York. Oxford.

Aloni, B.; Eitan, A. and Livne, A. (1977): The erythrocyte membrane site for the effect of temperature on osmotic fragility. Biochemica et Biophysica Acta. 465: 46-53.

Benjamin, M.M. (1984): Outline of Veterinary Clinical Pathology. 3rd. ed. The Iowa State Univ. Press, Ames, Iowa, USA.

Blechner, J.N. (1960): Sodium and potassium in the erythrocyte of adult and foetal goats. Amer. J. Physiol. 199: 1174-1176.

Blechner, J.N. (1961): Ionic composition of red cells in foetal, newborns and adult sheep. Amer. J. Physiol. 201: 85-88.

Brewer, G.J. (1974): The Red Blood Cell. Vol. 1 (Edited by Surgenor D.Mc.). Academic Press, New York.

Brown, C.G.D. (1990): Theileriosis: In Handbook on Aninal Diseases in the Tropics, 4th. ed.(Eds M.M.H. Sewell & D.W. Brocklesby) pp. 183-199. London, Bailliere, Tindall.

Burstein, S.A. and Harker, L. A. (1981): Quantitative platelet disorders. In: Haemostasis and Thrombosis. Bloom, A.L. and Thomass, D.P.(eds.): Edinburgh, Churchill Livingstone.

Christinaz, P. and Schatzman, H.J. (1972): High potassium and low potassium erythrocytes in cattle. J. Physiol. Lond., 224 : 391-406.

Clegg, M.N.; Keen, C.L.; Lonnerdal, B. and Hurley, L .S. (1981): Influence of ashing techniques on the analysis of trace elements in animal tissues. J. Wet Ashing Trace Element Res., 3: 107.

Coles, E.H. (1986): Veterinary Clinical Pathology: cerebrospinal fluid. W. B. Saunders Company, pp.: 267-278.

El- Metenawy, T. M. (1996): Epidemiological studies on tropical theileriosis at Al-Qassim area in Saudi Arabia. J. Egypt. Vet. Med. Assoc., 56 (2): 177-185.

El- Metenawy, T. M. (2000): Prevalence of blood parasites among cattle in the central area of Saudi Arabia. Vet. Parasitol., 87: 231-236.

Feldman, G.; Gagnon, J.; Hoffman, R. and Simpson, K.J. (1994): Statriev V.4.01. The solution for data analysis and presentation graphics. Abacus Lancripts Inc., CA, USA.

Fleck, S. L. and Moody, A. H. (1993): Diagnostic Techniques in Medical Parasitology. Butterworth & Co. (Publishers) Ltd.

Giardina, S.P. and Bretana, A.Y Marquez Q.N. (1983): Ultrastructural aspects of intra-erythrocytic development of a Venezuelan strain of *A. marginale*. Tropenmed. Parasit., 34: 7-10.

Hoogstraal, H. (1956): African Ixodoidca. 1. Ticks of the Sudan. Washington, D. C., PP.: 1101

Hoogstraal, H.; Wassef, H.Y. and Buttiker, W. (1981): Ticks (Acarina) of Saudi Arabia. Fam. Argasidae, Ixodidae. Vol. 3, pp.: 25-110.

Henry, J.B. (1984): Clinical Diagnosis and Management by Laboratory Methods, 17th. ed., W.B. Saunders Co. London.

Ibrahim, I. M.; Al-Nagedan, K.A.; Abdel- Rahman, H.S.; Hussein, S.A. and El-Mougy, S.A. (1994): Spleenectomy in the *Camelus Dromedarius*, 3- Erythrocytic sodium, potassium, magnesium, phosphorus and calcium changes. 2nd. Vet. Med. Cong. Zagazig, 11-13 October, Egypt: 55-61.

INRA. (1978): Alimentation des Ruminants, INRA publications. Versailles, France.

Levine, N.D. (1985): Veterinary Protozoology. Iowa State University Press, Ames, USA.

Magzoub, M.; Omar, O. H.; Haroun, E.M. and Mottelib, A. A. (1992): An outbreak of infections among cattle at Al-Qassim area of Saudi Arabia. Assiut Vet. Med. J., 28 (55): 206-214.

Moore, D.J. (1979): Disseminated intravascular coagulation: a review of its pathogenesis, manifestations and treatment. J. S. Afr. Vet. Assoc., 50: 259-264.

Mulei, C.M. and Daniel, R.C.W. (1988): Effect of age on erythrocyte magnesium, sodium and potassium concentration in female dairy cattle. Vet. Res. Commun., 12: 113-118.

Nordelo, M.A. and Ysern-Caldentey, M. (1982): Abnormal bovine erythrocyte membrane proteins and glycoproteins during and after infection with *Anaplasma marginale*. Biochem. Biophys.Res. Commun., 104: 664-672.

Radostits, O.M. Blood, D.C. and Gay, C.C. (1994): Veterinary Medicine. (8^{th}-ed.). Elbs. Bailliere Tindall, London.

Reece, W.O. (1992): Physiology of Domestic Animal, 2 ed., Williams & Wilkins A Waverly Co. London.

Ruckebusch, Y.; Phaneuf, L. pH. And Dunlop, R. (1991): Physiology of Small and Large Animals. B. C. Decker. Inc., Philadelephia. Hamilton.

Sandhu, G.S.; Grewal, A.S.; Singh, A.; Kondal, J.K.; Singh, J. and Brar, R.S. (1998): Haematological and biochemical studies on experimental *Theileria annulata* infection in crossbred calves. Vet. Res. Commun., 22(5): 347-354.

Schroeder, W. and Ristic, M. (1968): Infectious Blood Parasites of Man and Animals. Vol. 1. Academic press, New York.

Silva, I. M.; Hubsch, C. and Ysern-Caldentey, M. (1989): Erythrocytic osmotic fragility and cation concentrations during experimentally induced bovine anaplasmosis. Comp. Biochem. Physiol., 94A (3): 455-459.

Soulsby, E.J.L. (1982): Helminths, Arthropods and Protozoa of Domesticated Animals. Baillirre, Tindall and Cassell Ltd. London.

Valentina, W.N. (1975): Metabolism of human erythrocytes. Archs. Intern. Med., 135: 1307-1313.

Table (1): Mean ± SE. of coagulation assays of normal and *Theileria* infected bulls.

Parameters	Control group	Infected group
PT sec.	21.0 ± 0.73	19.5 ± 2.13
Factor VIII sec.	36.33 ± 1.11	34.60 ± 1.10
APPT sec.	104.67 ± 3.80	82.00 ± 8.81*
Factor VIII sec.	49.00 ± 1.82	49.3 ± 1.88
Platelets count $10^3/\mu L$	420.00 ± 18.03	189.9 ± 24.15**

PT: Prothrombin time. **APPT:** Activated Partial Prothrombin time.

* $P < 0.05$ ** $P < 0.01$

Table (2): Hematological values in control and *Theileria*-infected bulls (mean and ± S.E.).

Parameters	Control group	Infected group
Hb g/dl	11.53 ± 0.56	9. 11 ± 0.40**
PVC%	31.73 ± 0.69	27.73 ± 1.70*
MCHC %	36.13 ± 1.14	34.14 ± 1.91

* $P < 0.05$ ** $P < 0.01$

Table (3): Cations concentrations of plasma (P) and intra-erythrocytic (E) in control and *Theileria*- infected bulls (mean and ± S.E.).

Parameters	type	Control group	Infected group
Na(mmol/L)	P	134.22±1.07	133.73±2.19
	E	56.26±1.88	69.96±16.76
K (mmol/L)	P	5.64±0.18	5.81±0.01
	E	54.77±8.30	53.40±1.97
Ca (mmol/L)	P	2.00±0.04	1.91±0.15
	E	12.53±2.15	12.18±1.72
Zn mmol/L	P	12.52±0.53	15.04±1.30
	E	240.69±13.28	229.16±12.14
Cu (mmol/L)	P	18.58±0.39	18.74±0.55
	E	96.06±2.83	105.12±5.12
Fe (mmol/L)	P	23.60±2.15	27.48±1.85
	E	5282.62±130.82	5492.59±351.79

* $P < 0.05$ ** $P < 0.01$

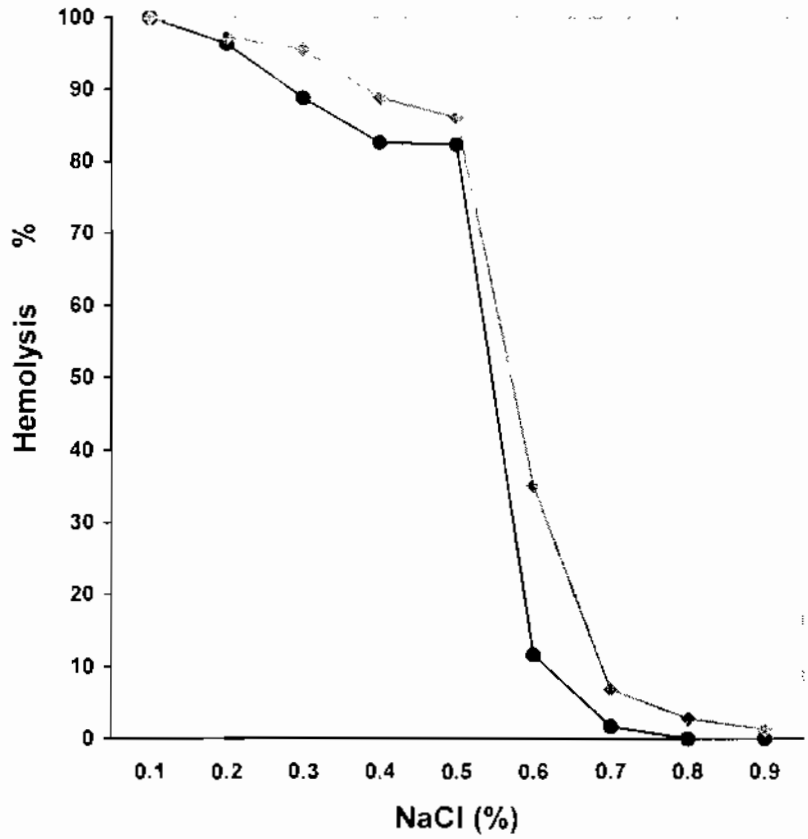

Fig. (1): Erythrocytic osmotic fragility of control (•) and infected (♦) bulls.

الملخص العربي

المقارنة بين منظور التجلط والهشهشة التناضحية للكريات الحمراء وتركيزات الشوارد في الماشية السليمة والمصابة بالثيليريا

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* قسم الطفيليات وامراض الحيوان- المركز القومي للبحوث- الدقى- مصر

بدراسة منظور التجلط وجد أن وقت البروثرومبين الجزئي النشط وعدد الصفائح الدموية قد نقص بصورة معنوية في المجموعة المصابة بالثيليريا عند مقارنتها بالمجموعة السليمة. لم يسجل أي تغير معنوي في مستويات الصوديوم - البوتاسيوم - الكالسيوم وكذلك العناصر النادرة (الزنك - النحاس - الحديد) بين العجول المصابة والسليمة سواء داخل أو خارج (البلازما) الكريات الحمراء. لقد وجد أن مستوى حجم الكريات المرصوصة والهيموجلوبين قد نقص بشكل ملحوظ في العجول المصابة . وقد وجد أيضا زيادة الهشهشة التناضحية للكريات الحمراء ب ٣ ، ٤ ، ٣ أضعاف والهشهشة المسجلة في المجموعة السليمة عند تركيزات ٨, ، ٧, ، ٦,% من كلوريد الصوديوم على الترتيب.