Studying the correlations among hematological and serum biochemical constituents in cattle theileriosis

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Abstract The present study was undertaken to investigate the influence of tropical theileriosis on serum constituents, erythrocytes and platelets pictures and to study the correlation between the studied parameters. A total number of 26 cows were subjected to study. Out of them 16 cows were suffered from theileria infection. Comparing theileria infected group with the control group revealed significant decreases in total RBCS counts ($p < 0.01$), haemoglobin concentration ($p < 0.01$), packed cell volume (PCV) ($p < 0.01$), platelets count (PLT) ($p < 0.01$), plateletcrit (PCT) ($p < 0.01$), significant decreases in serum total proteins ($p < 0.01$), albumin ($p < 0.01$), calcium ($p < 0.01$), and phosphorus ($p < 0.01$) levels, and significant increases in serum blood urea nitrogen (BUN) ($p < 0.05$) and creatinine ($p < 0.05$) levels, and in serum aspartate aminotransferase ($p < 0.01$) and gamma glutamyl transferase (GGT) ($p < 0.01$) activities. Correlations between serum biochemical parameters revealed significant positive correlations between total proteins and albumin ($r = 0.598^*$), A/G ratio and albumin ($r = 0.860^{**}$), calcium and albumin ($r = 0.729^*$), calcium and A/G ratio ($r = 0.752^*$), GGT and BUN ($r = 0.539^*$), and significant negative correlations between A/G ratio and globulins ($r = -0.809^{**}$) and between glucose and albumin ($r = -0.614^*$). Erythrocytes count showed a significant negative correlation with mean corpuscular volume (MCV) ($r = -0.966^{**}$) and red blood cells distribution width (RDW) ($r = -0.909^{**}$). MCV showed a significant positive correlation with RDW ($r = 0.860^*$) and PCV ($r = 0.781^*$). RDW was

positively correlated with PCV ($r = 0.966^{**}$). PLT count showed a significant positive correlation with mean platelet volume (MPV) ($r = 0.992^{**}$), platelets distribution width (PDW) ($r = 0.956^{**}$) and PCT ($r = 0.994^{**}$). Furthermore, MPV showed a significant positive correlation with PDW ($r = 0.940^{**}$) and a negative correlation with PCT ($r = -0.974^{**}$). PDW was negatively correlated with PCT ($r = -0.974^{**}$). It could be concluded that theileria infection in cattle resulted in mia, thrombocytopenia, hypoproteinaemia, hypoalbuminaemia and hypophosphataemia, in addition to hepatic and kidney dysfunction.

Keywords *Theileria* · Cow · Platelets · Erythrocytes · Serum

Introduction

Tropical theileriosis is one of the most prevalent and economically important diseases of cattle. The principle causative agent of bovine theileriosis in Egypt is the protozoan parasite *Theileria annulata*, which is transmitted by the ticks of the genus *Hyalomma*. The disease is observed in South Europe, North Africa, middle and South Asia and the Middle East and threatens approximately 250 million cattle (Salih et al. 2007). Theileriosis causes serious economic losses through mortality and loss of productivity (Glass et al. 2003; Aktas et al. 2004). *T. annulata* sporozoites infects the host mononuclear cells in the lymph nodes draining the site of inoculation of the sporozoites by ticks. The sporozoites transform into schizonts in the mononuclear cells. Host cells become transformed and proliferate in synchrony with the parasite during the macroschizont stage (Graham et al. 2001). The schizonts

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undergo further differentiation to merozoites, which are released by the lyses of the infected cells. Later, the merozoites invade red blood cells. It is followed by the development of piroplasms in erythrocytes, and these intra-erythrocyte piroplasms become available to the vector (Campbell and Spooner 1999; Forsyth et al. 1999).

Platelets (PLT) are anucleate cells with no DNA, derived from their precursor, the megakaryocyte, whose differentiation is characterized by nuclear polyploidization through a process called endomitosis (Van der Loo and Martin 1997). At present, the complete blood cells count can be performed using an automated hematology analyzer, which can increase the throughput of the test. Recently, new indices related to erythrocytes, and platelet counts have been provided by hematologic analyzers (Lombarts et al. 1986).

Concerning the platelet parameter, the three important parameters are plateletcrit (PCT), mean platelet volume (MPV) and platelets distribution width (PDW). Little is documented in the literature regarding the clinical interpretation of these parameters in cattle theileriosis. MPV is the best known of those three parameters. Platelet volume is a marker of platelet function and activation (Threatte 1993). In very general terms, increased MPV might be expected in “regenerative” thrombocytopenia, i.e., that caused by increased peripheral loss, destruction, or utilization of platelets and accompanied by increased production of platelets by marrow (megakaryocytic hyperplasia) (Bath and Butterworth 1996). PCT is a measurement derived from the platelet count and the MPV. PDW is a measurement derived from direct flow cytometric measurement of platelet cell volume, are less documented for their clinical roles (Lombarts et al. 1986; Van der Loo and Martin 1997).

The severity of infection with theileriosis is indicated by the change in blood serum biochemical parameters. Based on the above consideration, the present study was designed to study the influence of the tropical theileriosis on some serum components, erythrocytes and platelets pictures and to investigate values for PLT count, PCT, MPV and PDW and the correlation with the parallel red blood cell parameters in cattle Theileriosis.

Materials and methods

Animals

A total number of 26 cows were subjected to study. Out of them, 16 cows were presented to the Veterinary Teaching Hospital, Assiut University, showed high fever (40–41.5 °C), enlargement of superficial lymph nodes, and infestation with ticks, some cases showed corneal opacity and lacrimation, and

represented the theileria infected group. The remained animals ($N = 10$) were selected from healthy cows and kept as the control group. Theileria infection was confirmed by blood smear.

Samples

Drop of blood from the ear vein was used for making blood film according to Coles (1986). Blood smears were fixed in absolute methyl alcohol, stained with Giemsa stain and examined under the oil immersion lens (100×).

Two blood samples were collected from the jugular vein; the first blood sample was collected in Vacutainer tube (Becton–Dickinson, Rutherford, NJ) containing EDTA as anticoagulant and used for hematological analysis. The second blood sample was collected in plain vacutainer tube (Biomedica Alex Co., Egypt) and used for obtaining serum. Haematological analysis was carried out by means of Veterinary Haematology Analyzer (Medonic CA 620, Sweden). Serum samples were used to measure serum total proteins, albumin, blood urea nitrogen (BUN), creatinine, calcium, phosphorus and glucose levels, and serum aspartate aminotransferase (AST, U/l), gamma glutamyl transferase (GGT, U/l) and creatine phosphokinase (CK, U/l) activities using commercial kits (Spinreact, GIRONA, Spain) and by means of VIS/Ultraviolet Spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea).

Statistical analysis

Data were presented as mean and standard deviation. Statistical significance was determined by the analysis of variance using statistical package for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA). The correlations between red blood cell and platelet parameters and between the biochemical parameters were assessed using Pearson’s correlation analysis. Statistically significant differences were determined at $p \leq 0.05$.

Results

Blood smear

Beside clinical signs of theileriosis, diagnosis of theileria infection was confirmed by detecting intracellular signet ring of theileria trophozoites in blood smear (Fig. 1).

Haematological findings

As shown in Table 1, there were significant decreases in total RBCS counts ($p < 0.01$), haemoglobin (Hb)

Fig. 1 Blood film showing intracellular signet ring of theileria trophozoites

Table 1 Values of haematological parameters in diseased cows

	Control group	Theileria infected group
Total RBCs count ($\times 10^6$)/mm ³	7.59 $\pm$ 0.78	4.47 $\pm$ 0.35**
Haemoglobin (g/dl)	11.43 $\pm$ 0.51	6.38 $\pm$ 0.10**
PCV %	36.66 $\pm$ 1.52	17.98 $\pm$ 0.46**
MCV (fl)	48.53 $\pm$ 3.54	40.44 $\pm$ 3.88
RDW (%)	26.96 $\pm$ 1.22	31.78 $\pm$ 6.29
MCH (pg)	15.90 $\pm$ 0.70	14.34 $\pm$ 1.21
MCHC (%)	32.93 $\pm$ 1.85	35.58 $\pm$ 0.42
Total WBCs ($\times 10^3$)/mm ³	11.10 $\pm$ 0.40	12.24 $\pm$ 2.73
PLT ($\times 10^3$)/mm ³	461.0 $\pm$ 107.61	178.80 $\pm$ 106.32**
MPV(fl)	6.30 $\pm$ 0.17	6.16 $\pm$ 0.59
PDW %	10.70 $\pm$ 0.30	10.28 $\pm$ 1.43
PCT %	0.29 $\pm$ 0.06	0.10 $\pm$ 0.04**

Data expressed as mean $\pm$ SD, ** $p < 0.01$

concentration ($p < 0.01$), packed cell volume (PCV) ($p < 0.01$), PLT count ($p < 0.01$), PCT ($p < 0.01$) in theileria infected group when compared with the control group (Table 1).

Correlation between RBCs and platelets indices in theileria infected groups

Erythrocytes count showed a significant negative correlation with MCV ($r = -0.966^{**}$) and RDW ($r = -0.909^{**}$). MCV showed a significant positive correlation with RDW ($r = 0.860^*$) and PCV ($r = 0.781^*$). RDW was positively correlated with PCV ($r = 0.966^{**}$). PLT count showed a significant positive correlation with MPV ($r = 0.992^{**}$), PDW ($r = 0.956^{**}$) and PCT ($r = 0.994^{**}$). Furthermore,

MPV showed a significant positive correlation with PDW ($r = 0.940^{**}$) and a negative correlation with PCT ($r = -0.974^{**}$). PDW was negatively correlated with PCT ($r = -0.974^{**}$) (Table 2).

Serum biochemical findings

There were significant decreases in serum total proteins ($p < 0.01$), albumin ($p < 0.01$), calcium ($p < 0.01$) and phosphorus ($p < 0.01$) levels. In addition, there were significant increases in serum BUN ($p < 0.05$) and creatinine ($p < 0.05$) levels, and in serum AST ($p < 0.01$) and GGT ($p < 0.01$) activities (Table 3).

Correlations between serum biochemical parameters

Correlations between serum biochemical parameters revealed significant positive correlations between total proteins and albumin ($r = 0.598^*$), A/G ratio and albumin ($r = 0.860^{**}$), calcium and albumin ($r = 0.729^*$), calcium and A/G ratio ($r = 0.752^*$), GGT and BUN ($r = 0.539^*$), and significant negative correlations between A/G ratio and globulins ($r = -0.809^{**}$) and between glucose and albumin ($r = -0.614^*$) (Table 4).

Discussion

The tick-born protozoan disease, theileriosis, poses important problem for the health and management of domestic cattle in the tropics and subtropics. In Egypt, bovine theileriosis represents an important obstacle for the development of the livestock industry through the losses of milk and meat production.

The quantitation of platelets in peripheral blood is a well-recognized tool. However, other platelet parameters that recently have become available on a routine basis with the introduction of automated cell analyzers may become increasingly important in evaluating the integrity of the thrombocytic function (Bain 1985; Mohr et al. 1986; Van der Lelie and Von dem Borne 1986; Graham et al., 1987).

Haematological findings of the current study revealed significant decreases in total RBCs count, Hb concentration, PCV, PLT and PCT, which indicated severe anaemia, the same was reported by Omer et al. (2002). The results also agreed with that of Sandhu et al. (1998) who reported normocytic normochromic anaemia in cross-bred calves experimentally infected with *T. annulata*. The significant decrease in PCT ($p < 0.01$) is attributed to the significant thrombocytopenia ($p < 0.01$) (Schalm et al. 1975), thrombocytopenia together with the normocytic normochromic anaemia may indicate that anaemia may be partially attributed to depression of the bone marrow. The

Table 2 Correlation between RBCs and platelets indices in diseased cows

	RBCs count ($\times 10^6$) (mm ³)	MCV (fl)	RDW (%)	PCV (%)	PLT count ($\times 10^3$)/mm ³	MPV (fl)	PDW (%)	PCT (%)
RBCs count ($\times 10^6$)/mm ³	1	−0.966**	−0.909**	−0.593	−0.345	0.283	0.592	−0.437
MCV (fl)		1	0.983**	0.781*	0.095	−0.026	−0.364	0.196
RDW (%)			1	0.860*	−0.041	0.122	−0.220	0.066
PCV (%)				1	−0.544	0.604	0.298	−0.452
PLT count ($\times 10^3$)/mm ³					1	0.992**	0.956*	0.994**
MPV (fl)						1	0.940**	−0.974**
PDW (%)							1	−0.974**
PCT (%)								1

Significant (* $p < 0.05$); highly significant (** $p < 0.01$)

Table 3 Value of serum biochemical constituents in theileria infected cows

	Control group	Theileria infected group
Total proteins (g/dl)	6.79 $\pm$ 0.60	5.42 $\pm$ 0.45**
Albumin (g/dl)	3.56 $\pm$ 0.33	2.39 $\pm$ 0.45**
Globulins (g/dl)	3.23 $\pm$ 0.32	3.03 $\pm$ 0.40
A/G ratio	1.12 $\pm$ 0.09	0.81 $\pm$ 0.21*
BUN (mg/dl)	10.48 $\pm$ 1.57	17.57 $\pm$ 9.77*
Creatinine (mg/dl)	0.75 $\pm$ 0.26	1.52 $\pm$ 0.74*
Phosphorus (mg/dl)	6.58 $\pm$ 0.75	3.63 $\pm$ 1.06**
Calcium (mg/dl)	9.39 $\pm$ 0.95	6.56 $\pm$ 2.06**
Glucose (mg/dl)	76.47 $\pm$ 12.18	74.28 $\pm$ 30.62
GGT (U/l)	8.08 $\pm$ 2.06	20.84 $\pm$ 7.61**
AST (U/l)	30.01 $\pm$ 6.47	290.52 $\pm$ 36.79**
CK (U/l)	72.54 $\pm$ 34.22	91.65 $\pm$ 54.38

Data expressed as mean $\pm$ SD, * $p < 0.05$; ** $p < 0.01$

significant decrease in PCV may be attributed to oligocythaemia (Schalm et al. 1975) (Table 1).

RBCs count showed a significant negative correlation with MCV ($r = -0.966^{**}$) and RDW ($r = -0.909^{**}$), which may be attributed to the oligocythaemia that was associated with insignificant change in MCV and RDW. It is known that the change in MCV results in a change of PCV in the same direction. The positive correlations between MCV and PCV ($r = 0.781^*$) may be attributed to the significant decrease in PCV and the insignificant decrease in MCV. The significant decreases in both PCT and PLT may constitute the cause that stand behind the significant positive correlations between PCT and PLT ($r = 0.994^{**}$).

Clinically, measurements of platelets volume have long been of interest to researchers concerned with platelet production. MPV correlates with platelet function and activation, whether measured as aggregation, thromboxane synthesis, beta-thromboglobulin release, procoagulant

function, or adhesion molecule expression (Bath and Butterworth 1996). Concerning PCT, there is evidence supporting that PCT, rather than platelet counts, predicts the risk of bleeding in patients with thrombocytopenia (Mohr et al. 1986). PDW is a quantitative assessment of platelet size and volume and it is of limited usefulness in distinguishing between reactive thrombocytosis and essential thrombocythemia. PDW is increased in the presence of platelet anisocytosis (Van der Lelie and Von dem Borne 1986).

In the present study, theileria infected group showed hypoproteinaemia ($p < 0.01$) and hypoalbuminaemia ($p < 0.01$), which may be attributed to liver damage. The same was reported by Omer et al. (2003). The elevation in serum AST ($p < 0.01$) and GGT ($p < 0.01$) activities (Table 3) in the theileria infected group support the occurrence of hepatic dysfunction. The elevation in serum AST in cattle theileriosis was reported by Saber et al. (2008). Sandhu et al. (1998) reported that *Theileria annulata* infection causes hepatic tissue damage that includes coagulative necrosis, distortion of hepatic cords and heavy infiltration of lymphocytes in the periportal areas, including severe damage to the hepatobiliary system. Not only the liver was affected by theileria infection, but the kidney function also disturbed as confirmed by the rise in serum BUN and creatinine levels. Sandhu et al. (1998) reported an increase in BUN and uric acid in calves infested with theileria, with focal to diffuse coagulative necrosis, severe damage to collecting tubules, haemorrhages and lymphoid aggregations in interstitial spaces. It has also been stated that parasitized lymphoid cells can infect non-lymphoid organs such as liver and kidney inducing tissues damage (Forsyth et al. 1999).

In the current study, theileria infected cattle showed hypophosphataemia and hypocalcaemia, the decrease in serum calcium level may be attributed to hypoalbuminaemia. It is important to mention that a high proportion of

Table 4 Correlation between serum biochemical constituents in theileria infected cows

	Albumin	Globulin	A/G ratio	BUN	Creatinine	Phosphorus	Calcium	Glucose	GGT	AST	CK
Total proteins	.598*	.455	.131	.359	.377	−.006	.350	−.290	.016	.287	−.164
Albumin	1	−.442	.860**	.052	.164	−.078	.729*	−.614*	−.416	.072	.070
Globulin		1	−.809**	.340	.235	.083	−.453	.357	.478	.240	−.260
A/G ratio			1	−.183	−.036	−.118	.752*	−.484	−.493	−.107	.134
BUN				1	.294	−.011	−.262	−.021	.539*	.337	.045
Creatinine					1	.247	−.051	−.294	−.108	.085	−.194
Phosphorus						1	−.566	.068	−.073	−.272	.141
Calcium							1	−.356	−.216	.190	−.258
Glucose								1	.287	−.245	.008
GGT									1	.155	.154
AST										1	.329

calcium is bound to albumin. However, it is the unbound calcium that is the most important physiologically. For this reason when albumin is low, the total calcium level may be misleading (Hamilton and Bickle 2006). The reduction in the serum phosphorus concentration may be due to the renal tubular defects. The same was reported by Sandhu et al. (1998). The non significant changes in CK activity indicate absence of muscle damage.

Correlations between the studied biochemical parameters in theileria infected group revealed significant positive correlations between total proteins and albumin ($r = 0.598^*$), A/G ratio and albumin ($r = 0.860^{**}$), calcium and albumin ($r = 0.729^*$), calcium and A/G ratio ($r = 0.752^*$), GGT and BUN ($r = 0.539$), and significant negative correlations between A/G ratio and globulins ($r = -0.809^{**}$) and between glucose and albumin ($r = -0.614$). The above correlations confirm the findings of the present study, for example the positive correlations between calcium and albumin may be attributed to the bound part of calcium which decreases with hypoalbuminaemia. The negative correlations between albumin and glucose may be attributed to the destruction of liver cells, which results in the release of glucose to the circulation and decrease albumin synthesis.

Conclusion

It could be concluded that theileria infection in cattle resulted in anaemia, thrombocytopenia, hypoproteinaemia, hypoalbuminaemia, and hypophosphataemia, in addition to hepatic and kidney dysfunction.

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