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IMPACT OF ANEMIA ON COAGULATION INDICES IN THEILERIOSIS INFECTED CATTLE

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Received: 18 January 2024; **Accepted:** 21 February 2024

ABSTRACT

The hemostatic mechanism is crucial to stop bleeding while ensuring that tissues receive enough blood flow. This investigation examined selected haematological and coagulation parameters in different degrees of anemic Theileriosis infected cattle and study correlation between RBCs and platelet indices. Haematological analysis indicated substantial declines ($p < 0.01$) in total RBCs counts, PCV% and (Hb) in different degrees of anemic Theileriosis infected cattle compared with control group. In severe anemic group, MCV showed a substantial rise ($p < 0.01$) compared with moderate anemic group and ($p < 0.05$) compared with mild anemic and control groups; while MCH showed a substantial increase ($p < 0.05$) compared with mild and moderate anemic groups. In the coagulation profile, Platelets count (PLT) and PCT% showed insignificant difference ($p > 0.05$) among anemic groups compared with control group. MPV and PDW demonstrated substantial decreases ($p < 0.01$) in all anemic groups when compared with control group. In moderate anemic group, Prothrombin time (PT) exhibited a substantial prolongation ($p < 0.01$) compared to control group and ($p < 0.05$) compared to mild anemic group. In severe anemic group, PT was significantly prolonged ($p < 0.05$) compared with control and mild anemic groups. Correlations between RBCs and platelet indices in mild, moderate and severe anemic Theileriosis infected cattle revealed significant correlations in all blood parameters. It could be concluded that different degrees of anemia caused by T. annulata infection in cattle are associated with marked changes in measured haematological and coagulation parameters.

Keywords: Hemostatic system, Coagulation, Prothrombin time, *Theileriosis*.

INTRODUCTION

Anemia is a common abnormal condition in ruminants. Pathological affection of none hemopoietic tissue is the main cause of anemia in cattle, rather than reflecting primary defects in erythropoiesis (Katsogiannou *et al.,* 2018). Anemia associate the later stage of severe diseases (Goddard and Leisewitz, 2010). Blood protozoa and endo- and ectoparasites are typically the cause of it (Radostits *et al.,* 2010 and Singh *et al.,* 2014). In anemic ruminants, pallor mucous membrane,

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intolerance to exercise, tachypnea or dyspnea, tachycardia and functional murmurs are the most common symptoms (Radostits *et al.*, 2010).

Bovine theileriosis is a tick-borne haemoprotozoan disease transmitted by Hyalomma spp. and caused by *Theileria annulata* (Sayin *et al.*, 2003). The diseased cow had swelling of the pre-scapular lymph nodes, fever, loss of appetite and lethargy, pale mucous membrane, drop in milk production and abortion in pregnant animals due to high fever (Radostits *et al.*, 2010). Symptoms of acute tropical theileriosis that are most commonly observed include nasal and ocular discharges with congestion of mucous membranes, coughing, and salivation (Osman and Al-Gaabary, 2007). Subcutaneous edema, corneal opacity, and diarrhea could be found in later stages of the disease (Reda, 2012).

Theileriosis-related anemia can arise from infections with schizonts (lymphocytes) or piroplasm (erythrocytic merozoites), or from a combination of both (Radostits *et al.*, 2010). According to Omer *et al.* (2002), anemia associated with theileriosis reported as macrocytic normochromic, macrocytic hypochromic or normocyctic normochromic anemia, while no literature was reported on microcytic hypochromic and hyperchromic.

Positive and negative regulators play a complicated role in hemostasis; an unbalanced response can result in either hyper coagulation (thrombosis) or hypo coagulation (haemorrhage), or both (Rebar *et al.*, 2005). The use of coagulation and haematological parameters in diagnosis, prognosis, and treatment is beneficial. These factors offer extremely useful information regarding the level of infection (Çöl and Uslu, 2006). Platelets count and coagulation tests are used to evaluate pathological changes of the hemostatic and coagulation systems to direct clinical therapy (Zhao and Lv, 2013). The usual components of a routine hemostasis testing profile are the total number of platelets, activated partial thromboplastin time (APTT), and prothrombin time (PT).

Platelets (PLT) are crucial component of hemostasis, thrombosis, and a variety of bleeding disorders, they are a nuclear cytoplasmic fragments of bone marrow megakaryocytes (Russell, 2010 and Boudreaux *et al.*, 2011). One well-known technique in veterinary diagnostics is platelets quantification in peripheral blood (Souza *et al.*, 2016). Productivity, consumption, sequestration, and loss all have an impact on the total number of platelets (Russell, 2010 and Boudreaux *et al.*, 2011).

The morphology and proliferation kinetics of platelets are correlated with platelets indices (PI), which are indicators of platelets activation. The platelet indicators that are most frequently evaluated are the platelet-large cell ratio (P-LCR), platelet distribution width (PDW), platelet volume (MPV), and plateletcrit (PCT) (Russell, 2010; Boudreaux *et al.*, 2011 and Ustundag Budak *et al.*, 2016). Cytokines (thrombopoietin, interleukin-6, and interleukin-3) are discovered to be related to the platelet volume (Larsen *et al.*, 2014). MPV levels rise in response to decreasing platelets synthesis because immature platelets grow larger and become more active. Greater MPV is correlated with greater platelet diameter, which is a sign of platelet activation and production rate (Ustundag Budak *et al.*, 2016).

Platelet anisocytosis increases PDW, which is a measure of volume variability in platelet size (Ustundag Budak *et al.*, 2016). There is a clear correlation between MPV and PDW under physiological conditions, and both often fluctuate in the same way (Vagdatli *et al.*, 2010). Conflicting reports regarding the link between platelet counts and volume have been found in the literature, indicating that they are affected by different mechanisms (Chandrashekar, 2013 and Mariani *et al.*, 2014).

PCT is the proportion of blood volume that platelets occupy (Chandrashekar, 2013). MPV and platelet counts are inversely correlated, although platelet mass is tightly controlled to maintain a consistent level (Margetic, 2012).

A laboratory screening test called prothrombin time is used to assess the coagulation factors of the common and extrinsic pathways to identify conditions affecting the activity of factors I, II, V, VII, and X (Chaudhry and Babiker, 2019). Veterinarians can benefit greatly from knowing the normal prothrombin time. Regarding coagulation tests like PT, APTT, D-Dimer, PLT, and fibrinogen, there is no gold standard in veterinary medicine that is comparable to the scoring system used in human medicine (Taylor *et al.*, 2001). Thus, the primary aims of this investigation were to examine the impact of varying degrees of anemia in cattle infected with theileriosis on prothrombin time and platelets indices, as well as the correlation between red blood cells and platelets indices in cattle that were anemic.

MATERIALS AND METHODS

I. Animals:

The research study, which took place from May 2021 to September 2022, involved 38 cattle of both sexes with various ages (ranging from 6 months to 5 years, according to the owner's information). On the principle of owner's complaints, animals were divided into two groups. A diseased group, consisting of 27 animals with theileriosis that were clinically anemic and exhibited characteristic symptoms. Animals were brought to the Clinics of Internal Medicine, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Assiut University, Egypt, from various villages around Assiut city. Clinical examination and laboratory confirmation using Giemsa staining of blood smears were used to diagnose the condition. 11 clinically healthy animals were obtained from a private Assiut farm to serve as the control group.

2. Clinical Examination:

Based on clinical symptoms, tick infestation and blood smear examination, theileriosis was diagnosed. Each animal had a clinical assessment, which includes inspection of skin and mucous membrane of the eye, measuring the body temperature, auscultation of lung, heart and rumen, palpation of superficial lymph nodes and examination of oral cavity. Symptoms of bovine theileriosis are noticed and documented.

3. Samples:

3.1. Blood samples:

To examine blood films, ear vein punctures were performed on each animal. The jugular vein was used to extract blood samples using an aseptic and dry needle. Two different types of whole blood samples were collected and they were kept cold until examination:

3.1. A. A whole blood sample was obtained using potassium salts of Ethylene Diamine Tetra-Acetic acid (K-EDTA) as an anticoagulant and used for complete blood count (CBC).

3.1. B. Whole blood samples were collected on sodium citrate 3.2% (1:9) for coagulation tests. At the laboratory; centrifugation was performed at 1500 rpm for 10 min for separation of citrated plasma that was used for prothrombin time (PT).

3.1. C. Blood films:

Thin blood films were prepared from blood samples obtained from the ear vein of animals. The slide was covered with just a drop of blood, which was then carefully smeared and let to air dry. The blood smear was fixed in absolute methyl alcohol and left to air dry for two minutes. Giemsa stain was applied in a diluted ratio (1:9) and left for 45 minutes, the smear was then rinsed in distilled water and allowed to air dry (Soulsby, 2005). After that, it was examined under a light microscope using an oil immersion lens (x1000).

4. Hematological Examination:

Within four hours of the samples being collected, a haematological examination was

completed using a three-part diff haematological analyzer model (MTC-3060). In particular, red blood cell count (RBCs), hemoglobin concentration (HGB), haematocrit (HCT), and total and differential leucocytic counts. Red blood cell indices include: main corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and red blood cell distribution width (RDW) were among the haematological variables that were measured. Total platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), large platelet concentration ratio (LPCR), and plateletcrit (PCT) were measured.

5. Coagulation profile:

5.1. Prothrombin time (PT):

Using an automated blood coagulation analyzer (TECO coatron® M1 coagulation analyzer, Germany) and a commercial test kit (Siemens healthcare diagnostics products GmbH, Germany), prothrombin time was measured on citrated plasma in accordance with the manufacturer's instructions.

6. Statistical analysis:

The mean and standard error of the data were shown. To assess statistical significance, an analysis of variance was performed using the statistical software for the Social Sciences for Windows (SPSS, version 10.0, Chicago, IL, USA). The associations between the features of red blood cells and platelets were assessed using Pearson's correlation analysis. Statistically significant differences were defined as $p \leq 0.05$ (Borenstein *et al.*, 1997).

RESULTS

A- Clinical findings:

Upon clinical examination, diseased cattle showed symptoms such as fever (39- 41°C), enlargement of superficial lymph nodes especially those in the prescapular and prefemoral regions, pale mucous membranes, accelerated heart and respiratory rates, nasal discharges, cough, ruminal atony, corneal opacity in some cases, tearing. Tick infestations in varying degrees were seen around the abdomen, base of the tail, udder, and groin area. In contrast, a clinical assessment revealed that the control group was both clinically and laboratory-healthy.

Blood smear

In addition to the clinical manifestations of theileriosis, the intracellular signet ring of theileria trophozoites in a blood smear was utilized to validate the diagnosis of *Theileria* infection (Fig. 1).

B-Hematological findings:

Classification of anemia depends on PCV%. Mild anemia (PCV=20-25 %), moderate anemia (PCV=10 -20%), severe anemia (PCV less than 10).

Table (1) demonstrated substantial declines ($p < 0.01$) in all anemic groups' packed cell volume (PCV%), haemoglobin (Hb) concentration, and total RBC counts when compared to the control group. Also, among anemic groups, notable declines ($p < 0.01$) were found except for Hb in moderate anemic, which had a substantial decline ($p < 0.05$) compared with mild anemic group.

In the severe anemic group, MCV exhibited a substantial increase ($p < 0.01$) when compared with the moderate anemic group and a substantial increase ($p < 0.05$) with mild and control groups, while MCH, displayed a substantial increase ($p < 0.05$) when compared with mild and moderate anemic groups. In mild and moderate anemic groups, MCV and MCH revealed negligible changes ($p > 0.05$) compared to the control group. MCHC, RDW-CV and RDW-SD in all anemic groups displayed negligible differences ($p > 0.05$) compared with the control group and between each other.

Total WBC counts, lymphocytes, monocytes, and granulocytes in the anemic groups didn't differ substantially ($p > 0.05$) from those in the control group or from one another.

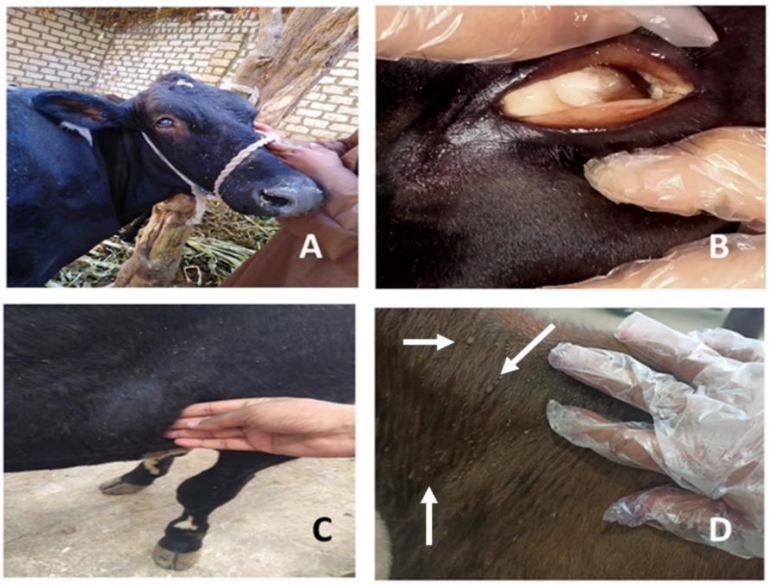

Figure (1): Cattle exhibiting clinical symptoms of Theileriosis: (A) opacity of the cornea; (B) paleness of the eye's mucous membrane and tearing; (C) swelling in the prefemoral L.N. (D) tick infestation on neck

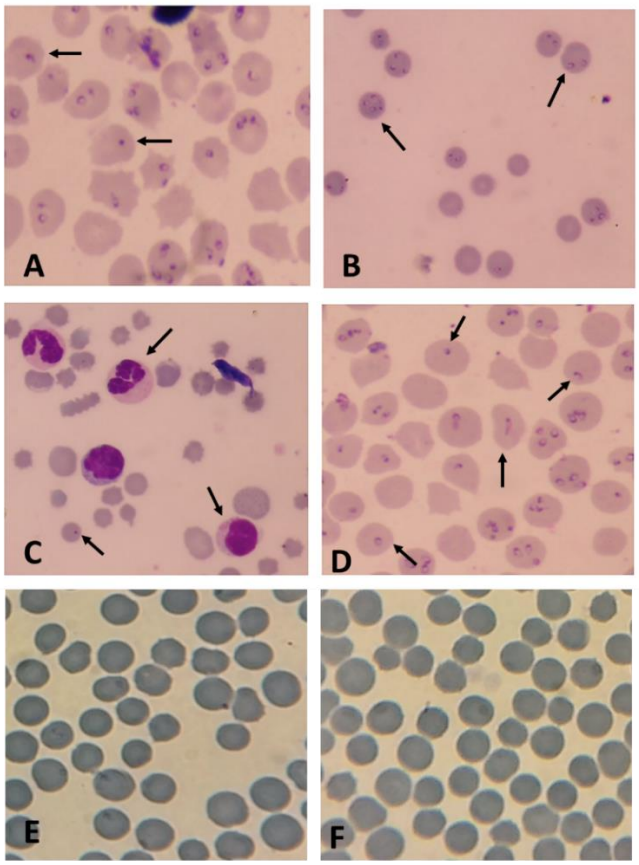

Figure (2): Giemsa-stained blood films from healthy control and Theilerosis infected cattle: (A, B and D) demonstrating poikilocytosis and anisocytosis of RBCs with single and multiple intra-erythrocytic signet ring, round, oval, dot, comma and rod-shaped piroplasms of *Theileria spp.* (C) demonstrating *Theileria* infected RBCs (dot shape), eosinophils and lymphocyte infected with *Theileria* parasites (Arrow referred to microschizonts); (E and F) Giemsa-stained blood films from healthy control cattle.

Table 1: Hematological parameters in control and anemic cattle with theileriosis (Mean ± SE).

Parameter	Control (n= 11)	Degree of anemia due to Theileriosis		
		Mild (n= 4)	Moderate (n= 18)	Severe (n= 5)
RBCs (10^6/µl)	7.44 ± 0.32 [a]	5.395 ± 0.42 [b]	3.64 ± 0.22 [c]	1.64 ± 0.298 [d]
MCV (fl)	46.64 ± 1.72 [b]	41.83 ± 4.27 [bd]	43.03 ± 1.39 [bc]	57.24 ± 7.97 [a]
MCH (pg)	16.85 ± 0.60 [ab]	14.18 ± 1.72[bd]	15.37 ± 0.53 [bc]	18.82 ± 1.69 [a]
MCHC (g/dl)	36.21 ± 0. 45 [a]	33.73 ± 0.88 [ad]	35.91 ± 0.50 [ab]	34.08 ± 1.89 [ac]
RDW-CV (%)	20.73 ± 0.49 [ac]	18.70 ± 1.25 [ad]	21.99 ± 0.54 [ab]	22.12 ± 3.98 [a]
RDW-SD (fl)	31.69 ± 1.26 [ad]	32.65± 6.70 [ab]	32.56 ± 2.23 [ac]	45.14 ±13.22 [a]
Hb (g/dl)	12.51 ± 0.58 [a]	7.43 ± 0.28 [b]	5.47 ± 0.24 [c]	2.90 ± 0. 26 [d]
PCV (%)	34.46 ± 1.45 [a]	22.03 ± 0.58 [b]	15.26 ± 0.68 [c]	8.48 ± 0.51 [d]
WBCs (10^3/µl)	10.04 ± 0.59 [ad]	14.23 ± 2.44 [a]	13.21 ± 2.16 [ab]	12.44 ± 2.63 [ac]
Lymph (10^3/µl)	6.12 ± 0.56 [ac]	9.33 ± 2.68 [a]	6.72 ± 1.68 [ab]	4.66 ± 1.13 [ad]
Mono(10^3/µl)	0.84 ± 0.16 [ad]	1.75 ± 0.25 [a]	1.55 ± 0.28 [ac]	1.60 ± 0. 30 [ab]
Gran(10^3/µl)	3.06 ± 0.22 [ad]	3.15 ± 1.00 [ac]	4.29 ± 1.04 [ab]	6.18 ± 1.60 [a]

Data expressed as mean ± SE, means of various superscript letters [a, b, c, d] in the same raw are substantially different at ($p < 0.05$), RBCs= Red blood cells, Hb=Hemoglobin, PCV=Packed cell volume, MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin, MCHC=Mean corpuscular hemoglobin concentration, WBCs= white blood cells, Lymph=Lymphocytes, Mono= Monocytes, Gran=Granulocytes

C- Coagulation profile findings:

Table (2) Comparing the anemic groups to the control group and to one another, platelets count and PCT% revealed negligible differences ($p > 0.05$). MPV and PDW demonstrated substantial decreases ($p < 0.01$) in all anemic groups when compared with the control group, but negligible changes ($p > 0.05$) were observed among the anemic groups. No discernible variations were seen between the groups in PLCC or PLCR.

In the moderate anemic group, PT exhibited a substantial prolongation ($p < 0.01$) when compared to the control group and a significant prolongation ($p < 0.05$) when compared to mild anemic group. In the **severe** anemic group, there was a significant prolongation ($p < 0.05$) when compared to the control and mild anemic groups. INR ratio showed that the severe anemic group had a significantly higher increase ($p < 0.05$) than the moderate anemic group.

Table 2: Coagulation parameters in control and anemic cattle with theileriosis (Mean ± SE).

Parameter	Control (n= 11)	Degree of anemia due to Theileriosis		
		Mild (n= 4)	Moderate (n= 18)	Severe (n= 5)
PLT (10^3/µl)	236.18 ± 22.29 [ac]	508.50 ± 120.85 [a]	401.83 ± 112.02 [ab]	216.00 ± 95.31 [ad]
PCT (%)	0.19 ± 0.02 [ac]	0.30 ± 0.06 [a]	0.24 ± 0.07 [ab]	0.13 ± 0.06 [ad]
MPV (fl)	8.14 ± 0.44 [a]	6.13 ± 0.38[bd]	6.41 ± 0.30 [b]	6.26 ± 0.19 [bc]
PDW (fl)	25.96 ± 4.71[a]	10.05 ± 1.67[c]	8.30 ± 0.62[cd]	10.28 ± 1.53[bc]
P-LCC (10^3/µl)	37.40 ± 5.27[ab]	27.67 ± 3.38[ac]	49.11 ± 15.57[a]	20.00 ± 9.89[ad]
P-LCR %	17.20 ± 3.16[a]	7.60 ± 2.58[ad]	16.19 ± 4.29[ab]	10.16 ± 1.57[ac]
PT (Sec)	20.42 ± 0.50[c]	19.25 ± 1.77[cd]	26.85 ± 1.37[ab]	28.78 ± 5.20[a]
INR ratio		0.94 ± 0.09 [bc]	1.32 ± 0.07 [ab]	1.41 ± 0.25 [a]

Data expressed as mean ± SE, means of various superscript letters [a, b, c, d] in the same raw are substantially different at ($p < 0.05$), PLT= platelets count, PCT = plateletcrit, MPV= mean platelet volume, PDW= platelets distribution width, P-LCC= platelet-large cell count, P-LCR= platelet-large cell ratio, PT= Prothrombin time, INR= International Normalized Ratio.

Association between platelets and RBCs indices in anemic cattle with theileriosis:

Table (3) in the mild anemic group, a notable negative association was found between the total number of RBCs and MCV (r = -0.961*), MCH (r = -0.981*), and MPV (r = -0.998**). MCV was substantially associated positively with MCH (r = 0.990**), RDW (r = 0.992**) and MPV (r = 0.970*). Also, MCH was considerably associated positively with RDW (r = 0.979*) and MPV (r = 0.982*). There was a notable positive association found between PLT count and PCT (r = 0.997**).

Table (4) in the moderate anemic group, the total number of RBCs displayed a notable opposite association with MCV (r = -0.703**), MCH (r = -0.718**), and RDW (r = -0.497*); nevertheless, there was a favorable association found between PCV (r = 0.848**) and Hb (r = 0.805**). A substantial positive association was observed between PCV and Hb (r = 0.935**). MCV was substantially associated negatively with MPV (r = -0.655**), but positively with MCH (r = 0.922**) and RDW (r = 0.737**). MCH exhibited a notable inverse association with MPV (r = -0.632**), while a favorable association was found with RDW (r = 0.616**). A notable positive association was observed between PLT count and PCT (r = 0.986**).

Table (5) in the severe anemic group, the total number of RBCs was substantially associated negatively with MCV (r = -0.895*) and MCH (r = -0.943*), but positively with Hb (r = 0.974**). MCV was substantially associated negatively with MCHC (r = -0.963**) but, positively with MCH (r = 0.983**). MCH had a noteworthy opposite association with MCHC (r = -0.910*). A substantial positive association was observed between RDW and MPV (r = 0.943*) and between PLT count and PCT (r = 1.000**).

Table 3: Association between platelets and RBCs indices in mild anemic cattle with theileriosis.

	RBCs count (10^6/µl)	PCV (%)	HB (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	PLT count (10^3/µl)	PCT (%)	MPV (fl)	PDW (fl)	PT (sec)
RBCs count (10^6/µl)		-0.348	-0.719	-0.961*	-0.981*	-0.779	-0.928	0.937	0.912	-0.998**	-0.159	-0.703
PCV (%)			0.749	0.583	0.519	0.153	0.673	-0.352	-0.387	0.372	-0.745	0.91
HB (g/dl)				0.797	0.824	0.768	0.852	-0.844	-0.876	0.709	-0.562	0.86
MCV (fl)					0.990**	0.669	0.992**	-0.881	-0.866	0.970*	-0.036	0.867
MCH (pg)						0.766	0.979*	-0.939	-0.925	0.982*	-0.026	0.821
MCHC (g/dl)							0.658	-0.941	-0.949	0.745	-0.073	0.428
RDW (%)								-0.869	-0.862	0.937	-0.157	0.918
PLT count (10^3/µl)									0.997**	-0.919	0.047	-0.66
PCT (%)										-0.893	0.118	-0.674
MPV (fl)											0.161	0.722
PDW (fl)												-0.47
PT (sec)												

The association is statistically substantial at $p < 0.05$ level (2-tailed) .*
The association is statistically substantial at $p < 0.01$ level (2-tailed).**

Table 4: Association between platelets and RBCs indices in moderate anemic cattle with theileriosis.

	RBCs count (10^6/µl)	PCV (%)	HB (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	PLT count (10^3/µl)	PCT (%)	MPV (fl)	PDW (fl)	PT (sec)
RBCs count (10^6/µl)		0.848**	0.805**	-0.703**	-0.718**	-0.183	-0.497*	0.324	0.39	0.259	0.323	-0.137
PCV (%)			0.935**	-0.238	-0.313	-0.258	-0.197	0.352	0.362	-0.151	0.166	-0.305
HB (g/dl)				-0.246	-0.184	0.098	-0.23	0.441	0.447	-0.159	0.106	-0.294
MCV (fl)					0.922**	-0.022	0.737**	-0.095	-0.197	-0.655**	-0.255	-0.149
MCH (pg)						0.364	0.616**	-0.026	-0.131	-0.632**	-0.304	-0.117
MCHC (g/dl)							-0.147	0.151	0.132	-0.025	-0.188	0.065
RDW (%)								0.032	-0.012	-0.317	-0.135	0.003
PLT count (10^3/µl)									0.986**	-0.219	-0.11	-0.256
PCT (%)										-0.08	-0.023	-0.228
MPV (fl)											0.251	0.269
PDW (fl)												-0.113
PT (sec)												

*. The association is statistically substantial at $p < 0.05$ level (2-tailed),
**. The association is statistically substantial at $p < 0.01$ level (2-tailed)

Table 5: Association between platelets and RBCs indices in severe anemic cattle with theileriosis:

	RBCs count (10^6/µl)	PCV (%)	HB (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	RDW (%)	PLT count (10^3/µl)	PCT (%)	MPV (fl)	PDW (fl)	PT (sec)
RBCs count (10^6/µl)		0.634	0.974**	-0.895*	-0.943*	0.826	-0.547	-0.537	-0.547	-0.443	-0.493	0.321
PCV (%)			0.765	-0.224	-0.359	0.14	0.285	0.151	0.147	-0.443	0.252	-0.442
HB (g/dl)				-0.789	-0.849	0.744	-0.357	-0.356	-0.365	-0.502	-0.303	0.195
MCV (fl)					0.983**	-0.963**	0.848	0.773	0.782	0.276	0.748	-0.675
MCH (pg)						-0.910*	0.776	0.771	0.779	0.259	0.683	-0.56
MCHC (g/dl)							-0.814	-0.664	-0.673	-0.322	-0.688	0.77
RDW (%)								0.842	0.849	0.095	0.943*	-0.784
PLT count (10^3/µl)									1.000**	-0.333	0.683	-0.712
PCT (%)										-0.316	0.694	-0.713
MPV (fl)											0.303	0.207
PDW (fl)												-0.561
PT (sec)												

*. The association is statistically substantial at $p < 0.05$ level (2-tailed),
**. The association is statistically substantial at $p < 0.01$ level (2-tailed)

DISCUSSION

Data from published works indicates that anemia is typically the primary clinical symptom of theileriosis and the severity of the condition depends on the species of theileria and the degree of parasitemia (Stockham *et al.,* 2000; Omer *et al.,* 2002 and Shiono *et al.,* 2004).

A–Clinical findings:

The clinical results seen in theileriosis-affected cattle in this investigation concurred with those reported by Kohli *et al.* (2014); Kachhawa *et al.* (2016); Gunes *et al.* (2017); Abd el-Hamied *et al.* (2020) and Yousef *et al.* (2020). The unchecked growth and spread of lymphoid cells infected with schizont, as well as the increased production of pro-inflammatory cytokines by parasitized monocytes, may be the cause of tissue damage and unfavorable clinical findings in cases of bovine theilerosis (Glass *et al.,* 2003 and Radostits *et al.,* 2010).

Cattle with bovine theilerosis may have fever as a result of activation of the thermoregulatory centre triggered by endogenous pyrogens released from cell lysis and parasitemia (Glass *et al.,* 2003). According to Radostits *et al.* (2010), anorexia may develop as a result of a persistently high fever during the acute phases of the disease. uncontrolled hyperplasia of T-lymphocytes infected with schizonts was the source of the generalized lymphoid proliferation that resulted in the enlargement of superficial lymph nodes (Radostits *et al.,* 2010). Leukocytic infiltration as a result of white blood cell infiltration may be the cause of corneal opacity in advanced cases (Hussein *et al.,* 2007 and Osman & Al-Gaabary, 2007). According to Muraguri *et al.* (2006) and Radostits *et al.* (2010), severe pulmonary edema that develops in advanced cases due to the release of vasoactive chemicals from disintegrating lymphocytes in the lungs may be the cause of the respiratory indications

that have been noticed, such as coughing, respiratory distress, and nasal discharge.

The etiology of anemia in theileriosis was thought to be extravascular hemolysis brought on by enzymatic, immune-mediated, mechanical, toxic, erythroid hyperplasia, and enhanced hemagglutinin processes (Stockham *et al.,* 2000 and Shiono *et al.,* 2003). Additional causes could be protease enzymes (Shiono *et al.,* 2004), oxygen radicals, elevated oxidative proteins in the membranes of red blood cells, and oxidative damage (Shiono *et al.,* 2003). Anemia and low haemoglobin concentration were indicated by pale mucous membranes as infected erythrocytes were distracted and removed by the reticuloendothelial system (Singh *et al.,* 2001).

B-Hematological findings:

According to reports from Singh *et al.* (2001) anemia can develop in the advanced stage of theileriosis after parasitaemia. Haematological results in this study demonstrated substantial declines in all anemic groups' packed cell volume (PCV%), haemoglobin (Hb) concentration, and total RBC counts compared to the healthy control group and amongst each other. The breakdown of erythrocytes by macrophages in the monocyte–macrophage system organs may be the cause of a drop in RBCs, PCV%, and Hb content (Singh *et al.,* 2001). According to Al-Emarah *et al.* (2012), the primary cause of the defects in erythrocytes that arise from parasite infections is their toxic activity. Anemia was reported by Haron *et al.* (2014) and might have been caused by erythrocyte lysis brought on by the parasite within the cells (Ghanem *et al.,* 2013 and Temiz *et al.,* 2014). The investigation's findings concurred with research conducted by Sarma *et al.* (2016); Lawrence *et al.* (2018); Nayak *et al.* (2018); Abd el-Hamied *et al.* (2020); Selim *et al.* (2020); Yousef *et al.* (2020); Nagar *et al.* (2021) and Aziz *et al.* (2022). Oligocythaemia could be the reason for the notable drop in PCV% (Abd Ellah, 2015).

Results of RBCs indices revealed a non-significant change ($p > 0.05$) in MCV and MCHC, showing normocytic normochromic anemia in mild and moderate anemic theileria-infected groups relative to the control group. The same was reported by Nazifi *et al.* (2010); Abd Ellah (2015); Ayadi *et al.* (2017)" in local and crossbred cattle" and Yousef *et al.* (2020).

The severe anemic group demonstrated a substantial increase ($p < 0.01$) in MCV and a negligible difference ($p > 0.05$) in MCHC compared to the control, mild and moderate anemic groups; this resulted in macrocytic normochromic anemia, which indicated a regenerative response consistent with a hemolytic anemia (Stockham *et al.,* 2000). The same was reported by Haron *et al.* (2014) and Devadevi *et al.* (2018).

The leucogram results showed that both relative to the control group and within the anemic groups, there were hardly detectable differences ($p > 0.05$) in the total WBC counts. The findings corroborated those of Ramin *et al.* (2011) and Temiz *et al.* (2014) who noted a negligible association between WBCs count and the severity of anemia. Also, the same was mentioned by Ganguly *et al.* (2015) and Ayadi *et al.* (2017) "in crossbred cattle".

The results contradicted the claims made by Qayyum *et al.* (2010); Khan *et al.* (2017) and Abubakar *et al.* (2019) regarding a decline in the total WBC counts. Conversely, several studies have found that the total WBC count in all theileria-infected animals increased significantly ($p < 0.05$) when compared to the controls. These studies include Temiz *et al.* (2014); Modi *et al.* (2015); Abd El-Hamed *et al.* (2016); Yousef *et al.* (2020) and Aziz *et al.* (2022). The chronic detrimental effects of Theileria's toxic metabolites on hemopoietic organs, particularly the bone marrow, and their disruption of the leucogenesis process may be the cause of changes in leucograms (Sarma *et al.,* 2016).

The current investigation found that the anemic groups' lymphocyte, monocyte, and granulocyte counts didn't have detectable variations ($p > 0.05$) relative to the control group or one another. The outcomes were comparable to those of Ramin *et al.* (2011) and Patel *et al.* (2017), who noted that the TLC and DLC (Neutrophil, Lymphocyte, Eosinophils and Monocyte) values fell within the standard range. Furthermore, Al-Emarah *et al.* (2012) noted that basophiles, eosinophils, and monocytes didn't exhibit a substantial statistical shift. In contrast, some studies referred to leucopenia and lymphopenia (Sandhu *et al.,* 1998) and others to lymphocytosis and leukocytosis (Stockham *et al.,* 2000 and Radostits *et al.,* 2010). According to Yamaguchi *et al.* (2010), lymphocytosis results from intra-lymphocytic theilerial parasites developing into host cells and clonally growing lymphocytes. According to Ganguly *et al.* (2015), there was a noteworthy rise in both monocytes and eosinophilic counts. This could perhaps be attributed to the multiplication of these cells as a host defense mechanism against infection, as documented by Modi *et al.* (2015) and Gunes *et al.* (2017). The leucocytic response is highest during acute infection and gradually declines during chronic and persistent infection, potentially explaining the variations in leucocytic profiles observed across different studies (Haron *et al.,* 2014).

C- Coagulation profile findings:

Platelets (PLT) and platelet indices are used in human medicine to diagnose a wide range of diseases and assess prognoses. However, the veterinary literature still has little to say about their clinical usefulness (Koenhemsi, 2019). There is evidence to suggest that in patients with thrombocytopenia, PCT, not platelet counts, predicts the risk of bleeding (Mohr *et al.* 1986). According to Bath and Butterworth (1996), MPV is linked to the function and activation of platelets concerning adhesion molecule expression, beta-thromboglobulin release, thromboxane production, aggregation, and procoagulant action. PDW is a measurable indicator of platelet volume and size. Platelet anisocytosis increases PDW. There is limited capacity to distinguish between essential thrombocythemia and reactive thrombocytosis using PDW (Van der Lelie and Von dem Borne 1986).

In the current study, platelets count and PCT% didn't significantly differ ($p > 0.05$) in anemic groups relative to the control group or one another. Reactive thrombocytosis could explain a non-statistically significant numerical rise in PCT% and platelets count in mild and moderate anemic groups. In the severe anemic group, a non-statistically significant drop in both PCT% and platelets count could be explained by bone marrow depression. MPV and PDW showed significant decreases ($p < 0.01$) in all anemic groups when compared with the control group, but there were no differences ($p > 0.05$) among the anemic groups.

The results support those of Modi *et al.* (2015), who found negligible changes in the platelet counts between theilerosis-infected cattle and the control group. Furthermore, concurring with Ravuri *et al.* (2020), who reported that reactive thrombocytosis patients had lower MPV and PDW than those with primary thrombocytosis. Additionally, in line with the findings of ten Berg *et al.* (2011) and Thon and Italiano (2012), who reported that thrombocytopenia brought on by decreased production rather than peripheral destruction has been demonstrated to reduce MPV and PDW.

Results contradicted those of Çöl and Uslu (2006); khan *et al.* (2017) and Devadevi *et al.* (2018) who noted a markedly lower platelet count in cattle infected with *Theileria* when compared to the control group. Additionally, in contrast to Abd Ellah (2015) who showed that in cattle infected with *Theileria*, despite a substantial decline in PCT% and platelet count, PDW and MPV didn't change. It is possible to attribute thrombocytopenia to either bone marrow suppression (Singh *et al.,* 2001) or the development of disseminated

intravascular coagulation (Maxie *et al.,* 1982).

Our findings partially align with those of Temiz *et al.* (2014) who discovered that anemic cattle infected with theilerosis had a substantial decrease in platelets count relative to the control group. However, there was no significant variation in platelet counts between the anemic groups. Also, with Memon *et al.* (2016) who found that in buffaloes infected with Theileria annulata, platelets and platelet indices did not change, Furthermore, with Gunes *et al.* (2017), who found negligible alterations ($p > 0.05$) in the MPV and platelet count in cattle with natural theileriosis, while the values of PDW and PCT were unchanged in healthy and infected groups. This suggests that the decline in platelet count may not be a reliable predictor of the illness's progression.

In a moderate anemic group of the current investigation, prothrombin time (PT) revealed substantial prolongation ($p < 0.01$) relative to the control group and ($p < 0.05$) relative to the mild anemic group; while in severe anemic group, there was a substantial prolongation ($p < 0.05$) relative to the control and mild anemic groups. INR ratio showed a substantial rise ($p < 0.05$) in the severe anemic group when compared with the mild anemic group. Hepatic damage, including localized necrosis, sinusoidal dilatation and disruption with haemorrhage and mononuclear cell infiltration on histological examination, can be used as an explanation for the prolongation of PT (Singh, 1998). These results are in line with those of Çöl and Uslu (2006) and Kilinc *et al.* (2018) who found that the prothrombin time of cattle infected with T. annulata was significantly longer ($P < 0.05$) than that of the control group. The coagulation abnormalities found in animals infected with theileria were also documented by Maxie *et al.* (1982) and Singh *et al.* (2001). The findings were in direct opposition to those of Gunes *et al.* (2017), who reported that there was no significant difference in (PT) between the control and infected groups in cattle having natural theileriosis. According to Hosny *et al.* (2010), the presence of parasites causes multiple petechial haemorrhages, lesions in the endothelium lining of blood vessels, and tissue damage in organs such as the liver, kidney, and lung. These outcomes are important because they contribute to the development of coagulation defects (Levi *et al.*, 1997).

In this investigation, the total number of RBCs displayed a notable opposite association with MCV ($r = -0.961^*$, $r = -0.703^{**}$ and $r = -0.895^*$) in mild, moderate and severe anemic groups, respectively, and with RDW ($r = -0.497^*$) in the moderate anemic group. There was a considerable positive association between MCV and RDW ($r = 0.992^{**}$ and $r = 0.737^{**}$) in mild and moderate anemic groups, respectively. Also, a notable positive association between PLT count and PCT ($r = 0.997^{**}$, $r = 0.986^{**}$ and $r = 1.000^{**}$) in mild, moderate and severe anemic groups, respectively was observed. Abd Ellah (2015) reported the same outcomes.

CONCLUSION

Notable alterations in the coagulation and haematological indicators have been linked with varying degrees of anemia in cattle caused by Theileria infection.

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تأثير الأنيميا على مؤشرات التجلط فى الأبقار المصابة بالثيليريا

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فقر الدم (الأنيميا) في الأبقار له أهمية كبيرة بسبب الخسائر الإقتصادية التى تتمثل فى فقدان أوزان الحيوانات وإنخفاض الإنتاج. تعتبر آلية تخثر الدم ضرورية لوقف النزيف مع ضمان حصول الأنسجة على تدفق دم كافٍ. أجريت هذه الدراسة على ٣٨ رأسا من الأبقارمن كلا الجنسين بأعمار تتراوح من ٦ اشهر الى ٥ سنوات. تم تقسيم الحيوانات إلى مجموعتين: تضمنت المجموعة الأولى ٢٧ حيواناً مصاباً بالأنيميا نتيجة الإصابة بداء الثيليريا وقد ظهرت عليها أعراض الإصابة بالمرض والتي تمثلت في إرتفاع في درجة الحرارة وضعف الشهية وتضخم الغدد اللمفاوية السطحية والهزال وشحوب الأغشية المخاطية وتدمع العينين وزيادة معدلات التنفس وضربات القلب كما وجد القراد على مناطق مختلفة من جسم الحيوّان المصاب. إشتملت المجموعة الثانية على ١١ حيواناً سليماً سريرياً. تم فحص عوامل التخثروعمل صورة دم كاملة للأبقار الخاضعة للدراسة، كما تم دراسة العلاقة بين مؤشرات كرات الدم الحمراء ومؤشرات الصفائح الدموية. أوضحت الدراسة أن داء الثيليريا فى الأبقار تسبب فى حدوث أنيميا بدرجات مختلفة وتم تقسيم الأنيميا بناءا على حجم خلايا الدم المرصوصة (PCV%) إلى ثلاث مجموعات (شديدة ومتوسطة وخفيفة). أشار تحليل الدم إلى إنخفاض معنوى (P <0.01) في إجمالي عدد كرات الدم الحمراء (RBCs) وحجم خلايا الدم المرصوصة (PCV%) وتركيز هيموجلوبين الدم (Hb) في مجموعات الأنيميا المختلفة بالمقارنة مع المجموعة الضابطة وبين بعضها البعض. **في مجموعة فقر الدم الشديد،** أظهر متوسط حجم خلايا الدم الحمراء (MCV) إرتفاعًا معنويا (p <0.01) بالمقارنة مع مجموعة فقر الدم المتوسط وإرتفاعا معنويا (p <0.05) بالمقارنة مع مجموعة فقر الدم الخفيف والمجموعة الضابطة؛ بينما أظهرمتوسط وزن الهيموجلوبين داخل خلايا الدم الحمراء (MCH) زيادة معنوية (P <0.05) بالمقارنة مع مجموعات فقر الدم الخفيف والمتوسط. كما لوحظ عدم وجود إختلافات معنوية (P > 0.05) فى تركيز الهيموجلوبين داخل خلايا الدم الحمراء (MCHC) والعدد الكلي لخلايا الدم البيضاء (WBCs) والخلايا الليمفية (Lymphocyte) والخلايا وحيدة النوا ة (Monocytes) والخلايا الحبيبية (Granulocytes) فى مجموعات الأنيميا المختلفة بالمقارنة مع المجموعة الضابطة وبين بعضها البعض. أظهرالعدد الكلي للصفيحات الدموية (PLT) وPCT٪ عدم وجود فروق معنوية (p > 0.05) بين مجموعات فقر الدم مقارنة بالمجموعة الضابطة وبين بعضها البعض، بينما أظهر معدل حجم الصفيحات (MPV) و معدل إنتشار الصفيحات (PDW) إنخفاضا معنوى (P <0.01) في جميع مجموعات فقر الدم بالمقارنة مع المجموعة الضابطة بينما لا توجد فروق معنوية بين بعضها البعض. **في مجموعة فقر الدم المتوسط ،** أظهر زمن البروثرومبين (PT) زيادة معنوية (P <0.01) مقارنة بالمجموعة الضابطة و (P <0.05) مقارنة بمجموعة فقر الدم الخفيف. بينما **في مجموعة فقر الدم الشديد،** ازداد زمن البروثرومبين (PT) بشكل ملحوظ (P <0.05) بالمقارنةً مع المجموعة الضابطة ومجموعة فقر الدم الخفيف. أظهرت النسبة الدولية المعيارية (INR) زيادة معنوية (P <0.05) في مجموعة فقر الدم الشديد بالمقارنة مع مجموعة فقر الدم الخفيف. كشفت الإرتباطات بين مؤشرات كرات الدم الحمراء ومؤشرات الصفائح الدموية في فقر الدم الخفيف والمتوسط والشديد للأبقار المصابة بداء الثيليريا عن وجود إرتباطات مهمة في جميع معايير الدم. خلصت الدراسة إلى أن فقر الدم الناجم عن الإصابة بداء الثيليريا في الماشية مرتبط بتغيرات ملحوظة في قياسات الدم وعوامل التخثر.

الكلمات الدالة: مؤشرات التجلط، ثيليريا، أنيميا، أبقار