

Research Article

Physiological study of cows infected with Theileriosis in Thi-Qar Governorate

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Abstract: This study was conducted in Thi-Qar Governorate to find out the effect of *Theileria annulata* parasite on the physiological status of red and white blood cells of cattle infected with *Theileria*. Parasitic infection was diagnosed based on PCR technique and clinical signs. The samples were divided into four groups: (G1) clinically uninfected with a negative PCR test result (the control group (n=30), (G2) a clinically infected group with a negative PCR test result (n=20), (G3) a group is clinically uninfected with a positive PCR test result (n=10), and (G4) clinically infected with a positive PCR test result (n=40). The results showed a significant decrease in the value of red blood cells, hematocrit, hemoglobin, red blood cells, and average hemoglobin. The results of the hematological criteria that included measuring the level of red blood cells (RBC) indicated that there were significant differences in the level of RBC in the second and third and fourth groups. The results revealed a significant decrease in the concentration of hemoglobin (Hb) and hematocrit (HCT) in the second, third, and fourth groups. The current study also recorded a significant decrease in the mean corpuscular volume (MCV) of red blood cells, whose value in the fourth group was 42.66 ± 1.62 compared with the control group. The results also showed a significant decrease in the values of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in the fourth group (11.59 ± 1.09 and 27.86 ± 2.05) compared with the control group (16.25 ± 0.81 and 33.15 ± 1.81). There were no significant differences in the values of red blood cell distribution width (RDW-SD) and the RDW-Red blood cell distribution width coefficient of variation (RDW-CV) in the fourth group (38.35 ± 5.82 and 16.49 ± 1.28) when compared with the control group (36.87 ± 2.95 and 15.35 ± 1.67). The current study showed a significant decrease in the white blood cells (WBC) in the third (9.3 ± 1.43) and the fourth (4.08 ± 1.34) groups compared to the control one (12.88 ± 1.915).

Keywords: *Theileria*, Theileriosis, Hematology, Parasite.

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Introduction

Theileria annulata is a blood parasite transmitted by ticks *Hyalomma* spp.. *Theileria annulata* is one of the most protozoan parasites that destroy blood components and cause anemia. The genus *Theileria* belongs to the Sporozoa and the family Piroplasmidia. *Theileria* parasite is the most important and the most common fatal disease and is economically important for livestock and is widely spread in Africa, southern Europe, the Near and Far

East, and Central Asia. Theileriosis also spread in most areas of Iraq and the disease had a significant economic impact on animal production due to the high rates of diseases and deaths (Ayadi et al. 2016), where Theileriosis is considered one of the most common diseases in Iraq, especially in the southern regions because the weather is hot and suitable for the middle host. The parasite damage causes anemia and an increase in the fragility of red blood cells (Nazifi et al. 2008). This damage leads to a loss of

autoimmunity, as it leads to wasting and malnutrition of cows. This disease affects livestock, as it causes the calves to not grow, thus delaying their growth and causing cows to produce less milk, in addition to poor quality meat (Ahmed et al. 2008). The most important symptoms of the disease Theileriosis are swollen lymph nodes, wasting, high temperatures (Hussein et al. 2004), sometimes diarrhea, weight loss, shortness of breath, and pallor of the mucous membranes (El-deeb Younis 2009). The aim of this study was to investigate some physiological changes that occur in some blood values as an indicator of the pathogenicity of the parasite *T. annulata* in infected animals compared with healthy animals.

Materials and methods

Areas of the study: The current study was conducted in Dhi Qar Governorate, which is located in the south of Iraq, and the focus was on the general veterinary hospital area, and blood samples were taken from cows randomly from both sexes and for all age groups. 214 blood samples were randomly collected from cows, taken from the jugular vein of cows after sterilizing the area with ethanol alcohol (70%) (Chaudhri & Gupta 2003), and 5ml of blood was withdrawn by a medical syringe (5ml) and distributed as follows: 2.5ml in tubes containing an anticoagulant substance (EDTA) to make fresh blood tests, which is used to measure red blood cells (RBC) and white blood cells (WBC) and to measure the concentration of hemoglobin (HB), hematocrit (HCT) and (2.5ml) and stored at -20°C for the purpose of extracting the DNA of the parasite *T. annulata*.

The samples were divided into 4 groups: (G1) a clinically uninfected group with a negative PCR test result (control group) (n=30), (G2) a clinically infected group with a negative PCR test result (n=20), (G3) is a clinically uninfected group with a positive PCR test result (n=10), and (G4) a clinically infected group with a positive PCR test result (n=40). The parasitic diagnosis was done

using the direct blood smear method according to Chaudhri & Gupta (2003). Also, DNA was extracted from blood samples that were collected randomly and by relying on the leaflet attached to the kit DNA extraction based on the manufacturer Geneaid/USA, according to Sambrook et al. (1989). PCR method was used to detect the parasite *T. annulata* and a pair of primers was used to amplify the cyto1 gene with a size of 312bp based on Yang et al. (2015) and as following primers: Forward: 5'-ACT TTG GCC GTA ATG TTA AAC-3', and Reverse: 5'-CTC TGG ACC AAC TGT TTG G-3'.

Clinical examination: The current study included 214 blood samples and recorded the most important clinical signs appearing on cows infected with *T. annulata*, represented by an increased temperature of 40.5°C, and enlarged lymph nodes in the shoulder area. Corneal yellowing and opacity, emaciation, increased respiratory rate, and yellowish diarrhea.

Blood examinations: Hematological parameters were measured using the automatic blood analyzer (GENEX COUNT 60, USA). The tube EDTA containing blood was placed under the needle that withdraw 150 microns and after about 53 seconds, the result of the examination including Red blood cell (RBC), hemoglobin (Hb), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin, red blood cell distribution width (RDW-SD), RDW- Red blood cell distribution Coefficient of Variation and RDW-CV, number of white blood cells (WBC), the percentage and number of lymphocytes %LYM and #LYM, the percentage and number of monocytes %MID and #MID, the percentage and number of neutrophils %NEUT and #NEUT, Platelet count (PLT), procalcitonin (PCT), platelet distribution width (PDW) and platelet volume (MPV) were obtained.

Results

The results showed the diagnosis of Theileria parasite in cows examined by the direct blood smear

Table 1. Shows the clinical signs of animals infected with *T. annulata* and diagnosed by smear method.

Clinical signs	Incidence in infected cows%
fever	10(24.4%)
mucous membranes	2(4.9%)
jaundice	4(9.8%)
lymph nodes	7(17.1%)
neurological	1(2.4%)
signs of Body condition (wasting, anemia, general weakness)	4(9.8%)
exophthalmos	3(7.3%)
Anemia	6(14.6%)
Diarrhea	1(2.4%)
Runny nose	3(7.3%)

method with a percentage of 19% (214/41) while in the PCR method, the infection rate was 50% (100/50) using the *cyto1* gene with a size of 312pb. The results also recorded the most important clinical signs in cows infected with *T. annulata* parasite, including exophthalmos, enlarged lymph nodes in the shoulder area, yellowing and opacity of the cornea, emaciation, increased respiratory rate, and yellowish diarrhea (Table 1).

The results showed an effect of parasitic infection in infected cows on red blood cells, where there were significant differences in the RBC value in the second (5.23 ± 1.41), the third (3.425 ± 0.75), and the fourth (2.946 ± 0.51) group compared with the control one (6.95 ± 0.42), where the fourth group (clinically infected) showed a significant decrease in RBC compared to the control group. No significant difference was between the third and fourth groups. The current study found a significant decrease in the concentration of Hb and HCT in the second group (10 ± 0.5 and 32.67 ± 2.52) in the third group (6.225 ± 0.97 and 26.23 ± 0.99), and the fourth group (6.362 ± 1.01 and 24.88 ± 1.80) compared to the control one (11.17 ± 0.76 and 33.13 ± 1.05). A significant decrease was found in Hb and HCT in the third and fourth groups compared to the second and control groups.

Also, a significant decrease in the MCV within the second (47.6 ± 1.9), third (44.25 ± 1.71), and fourth (42.66 ± 1.62) groups compared to the control group (48.78 ± 1.22) were observed. An insignificant

decrease was observed between the second and control groups with the third groups. The results showed a significant decrease in the values of MCH and MCHC in the fourth group (11.59 ± 1.09 and 27.86 ± 2.05) compared to the control (16.25 ± 0.81 and 33.15 ± 1.81) and the fourth group. MCH and MCHC of the second group were 11.59 ± 1.09 and 27.86 ± 2.05 , the second group 16.97 ± 0.57 and 33.4 ± 1.64 , the fourth group 11.59 ± 1.09 and 27.86 ± 2.05 and the third treatment 15.1 ± 1.92 and 33.4 ± 1.64 .

There were no significant differences for the RDW-SD and RDW-CV between the second (35.63 ± 1.99 and 16.57 ± 1.19), third (39.63 ± 2.49 and 16.68 ± 0.55), and fourth (38.35 ± 5.82 and 16.49 ± 1.28) groups compared to the control group (36.87 ± 2.95 and 15.35 ± 1.67) (Table 2). The results showed a significant decrease in WBC in the third (9.3 ± 1.43) and fourth (4.08 ± 1.34) groups compared to the control group (12.88 ± 1.915), and a significant decrease in MID%, the third (3.43 ± 0.33) and the fourth (3.59 ± 0.61) groups compared to the control group (3.59 ± 0.61), while there was a significant decrease in NEUT% in the third (25.5 ± 1.29) and fourth (24.74 ± 2.13) groups compared to the control one (27.5 ± 1.05). A significant decrease was found in the second group (16.83 ± 4.55) compared to the control group and a significant decrease in the lymphocytes in the third (5.68 ± 1.49) and fourth (5.45 ± 1.08) groups compared to the second group (17.1 ± 13.01). The results recorded no significant differences for MID and NEU in the second (1.3 ± 0.27 and 6.67 ± 0.58), third (1.4 ± 0.70 and $4.08 \pm$

Table 2. Effect of *T. annulata* parasite of bovines on red blood cells.

Parameters	RBC (10 ⁶ /ul) Mean±SD	HGB (g/dl) Mean±SD	HCT (%) Mean±SD	MCV (fl) Mean±SD	MCH (pg) Mean±SD	MCHC (g/dl) Mean±SD	RDW-SD (fl) Mean±SD	RDW-CV (%) Mean±SD
Groups								
G1	6.95±0.42 ^a	11.17±0.76 ^a	33.13±1.05 ^a	48.78±1.22 ^a	16.25±0.81 ^a	33.15±1.81 ^a	36.87±2.95 ^a	15.35±1.67 ^a
G2	5.23±1.41 ^{bA}	10±0.5 ^{aA}	32.67±2.52 ^{aA}	47.6±1.9 ^{aA}	16.97±0.57 ^{aA}	33.4±1.64 ^{aA}	35.63±1.99 ^{aA}	16.57±1.19 ^{aA}
G3	3.425±0.75 ^{cB}	6.225±0.97 ^{bB}	26.23±0.99 ^{bB}	44.25±1.71 ^{bB}	15.1±1.92 ^{aA}	33.68±2.47 ^{aA}	39.63±2.49 ^{aA}	16.68±0.55 ^{aA}
G4	2.946±0.51 ^{dC}	6.362±1.01 ^{cC}	24.88±1.80 ^{cC}	42.66±1.62 ^{cC}	11.59±1.09 ^{bB}	27.86±2.05 ^{bB}	38.35±5.82 ^{aA}	16.49±1.28 ^{aA}
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.654	0.296

The numbers in the table represent (Mean±S.D) values. Vertically: similar letters mean that there are no significant differences.

Table 3. Effect of the parasite *T. annulata* on white blood cells in cows.

Parameters	WBC (10 ⁶ /ul) Mean±S.D	%LYM Mean±SD	%MID Mean±SD	%NEUT Mean±SD	#LYM (10 ⁶ /ul) Mean±.D	#MID (10 ⁶ /ul) Mean±SD	#NEUT (10 ⁶ /ul) Mean±SD
Groups							
G1	12.88±1.915 ^a	60.2±5.36 ^a	5.26±0.59 ^a	27.5±1.05 ^a	7.13±0.57 ^a	1.38±0.23 ^a	6.07±1.14 ^a
G2	11.7±0.96 ^{aA}	60±4.36 ^{aA}	4.63±2.52 ^{aA}	16.83±4.55 ^{bA}	17.1±13.01 ^{bA}	1.3±0.27 ^{aA}	6.67±0.58 ^{aA}
G3	9.3±1.43 ^{bA}	57.75±2.21 ^{aA}	3.43±0.33 ^{bA}	25.5±1.29 ^{aB}	5.68±1.49 ^{aB}	1.4±0.70 ^{aA}	4.08±2.05 ^{aA}
G4	4.08±1.34 ^{cB}	55.13±3.22 ^{aA}	3.59±0.61 ^{cA}	24.74±2.13 ^{aC}	5.45±1.08 ^{aC}	1.45±0.74 ^{aA}	5.67±2.41 ^{aA}
P value	0.0001	0.06	0.0001	0.0001	0.0001	0.983	0.356

The numbers in the table represent (Mean±SD) values. Vertically: similar letters mean that there are no significant differences.

2.05), and fourth (1.45±0.74 and 5.67±2.41) groups compared to the control one (1.38±0.23 and 6.07±1.14) (Table 3).

Discussion

Effect of *T. annulata* parasite on erythrocytes in cows: The results of the current study showed a clear effect of infection with the parasite *T. annulata* on the level of red blood cells. A significant decrease in the groups infected with the parasite *T. annulata* (the third and fourth groups) compared to the control group, while the second group was uninfected with the parasite *T. annulata*, but it showed clinical symptoms similar to infection. This confirms the low value of RBC in this group due to infection with another disease. Al-Khafaji et al. (2011) conducted a study in Babil Governorate, where blood samples were taken from Cows and both sexes, where the value of red blood cells in infected cows was 10×6.50µm, while the value of red blood cells in healthy cows was 7.35×10µm, and

it was consistent with the result of the study conducted in Iran where 50 cows of 3-5 years old (Nazifi et al. 2009) and found RBC for infected cows as 3.95±0.07 compared with the control group as 6.64±0.23. Omer et al. (2002) mentioned in a study conducted in Saudi Arabia a decrease in the number of blood corpuscles. Erythrocytes oxidized red blood cells can be easily destroyed by erythrophagocytosis, in addition to the fact that macrophages in the lymph nodes and spleen destroy red blood cells (Singh et al. 2001).

Hemolytic anemia is one of the most important features of tropical theileriosis caused by immune hemolysis. Shiono et al. (2004) stated that the activity of antioxidant enzymes superoxide dismutase (SOD) is affected by the presence of *T. annulata*, it works on the fragility of red blood cells and their removal by phagocytes, where oxidizing factors work to convert hemoglobin to methemoglobin, which results in a decrease in oxygen, and thus oxidative damage is the cause of

anemia in bovine Theileria, the effect of merozoma (Shizonts), anti-erythrocytes antibodies change the red blood cells to different shapes and sizes, as hemolysis is proven by immunity (Muraleedharan et al. 2005). Ananda et al. (2009) and Ganguly et al. (2015) reported that the parasite multiplies inside red blood cells, which causes the destruction of the corpuscle causing hemolytic anemia.

The current study showed a decrease in the concentration of HB and HCT in the second and third and fourth groups; the results are consistent with Al-Emarah et al. (2012) which was conducted in Basra and the hemoglobin value of the affected cows was 6.95 ± 0.59 compared to the control group (10.93 ± 1.03). Our results were consistent with Al-Khafaji et al. (2011), where the hemoglobin value of the infected cows was 10.68g/dL compared with the control one 11.78g/dL that the cause of anemia due to the ingestion of red blood cells containing the infectious phase pyroplasm by phagocytic cells in Reticulo-endothelial system (Reticulo endothelial system). Boulter & Hall (2000) mentioned that tumor necrosis factor- α (NTF- α) is the cause of anemia and hemolytic anemia is caused by the hemolytic immune system (Aulakh et al. 1998). Low hemoglobin may occur due to severe infection of red blood cells that have been removed by the body's immune system (Alsaad et al 2013).

Some studies' results agree with the results of the current study on decreasing HCT (Qayyum et al. 2010; Khan et al. 2011; Al-Emarah et al. 2012; Omer et al. 2002). Qayyum et al. (2010) and Khan et al. (2011) observed a decrease in hematocrit in cows infected with *T. annulata*, as a result of a decrease in hemoglobin (hemoglobin), which leads to anemia, which leads to a decrease in the value of stressed cells. The RBC is directly proportional to the hematocrit value, and then the anemia resulting from the increase in the number of RBC infected with pyroplasm, which leads to the removal and destruction of red blood cells from the hematocrit, leads to a decrease in the hematocrit value (Kulkarni

2011).

The current study recorded a significant decrease in MCV due to infection with *T. annulata* in the clinically infected group (the fourth group) and the clinically uninfected group (the third group) compared to the control group. This result is consistent with the study of Abubakar et al. (2019), where the average value of red blood cells recorded in cows infected with *T. annulata* was 57.6 ± 6.5 , while it differed from the study conducted in Basra by Al-Emarah et al. (2012) in which the value of the average red blood cell volume was higher than it was before the injury, that these results are due to anemia that leads to a decrease and destruction of blood corpuscles. Red blood as a result of chromosomal anemia in the final stages of the disease Abubakar et al. (2019) while Sandhu et al. (1998) showed that normal anemia in cattle infected with *T. annulata* parasite occurs at later stages of infection.

The current study showed a significant decrease in the value of MCH in the fourth group compared with the control and between the fourth and second groups and the fourth and third groups, and this is consistent with Al-Emarah et al. (2012), and the reason for the decrease was as a result of the direct feeding of ticks on blood in that it is a permanent bloodsucker (Durrani et al. 2008). The current study showed a significant decrease in MCHC in the fourth group compared with the control and between the fourth and second groups and the fourth and third groups, and a decrease in the values of MCHC in the affected cows. These results were consistent with Al-Emarah et al. (2012) where the average value of hemoglobin in the affected cows was 24.71 ± 4.55 and in the control group its value was 32.32 ± 2.67 ; it occurs due to normal chromosomal anemia (Alsaad et al. 2013).

The study found no significant differences in the RDW-SD and RDW-CV between the three groups compared to the control group and between the second, control, and third groups, as it was inconsistent with Omer et al. (2002) and the ratio of

RDW-SD was 0.516 ± 22.5 in infected cows 0.381 ± 22.2 and in a study conducted by Gunes et al. (2017) that the value of RDW-SD had significant differences in uninfected cows (16.32 ± 0.18).

Effect of the parasite *T. annulata* on white blood cells in cows: The current study showed a decrease in white blood cells in the two groups diagnosed by PCR; it was consistent with the results of Abdel-Hamied et al. (2020). The reason for the decrease in white cells is due to the change in those cells and their decrease in the last stages of the disease as a result of the continuous and harmful effects that are caused by the *T. annulata* on the lymphatic organs, especially in places of blood formation such as the bone marrow and that the destruction of cells Lymphatics through parasite proliferation in the lymphatic organs, which leads to a decrease in white blood cells (Ibrahim et al. 2009). The results of the current study showed a decrease in the value of lymphocytes, as these results were consistent with Omer et al. (2002) and the decrease is in these cells in lymphocytes as a result of their destruction and parasite proliferation (Col & Uslu 2007). The results showed a decrease in the value of monocytes, as this study agreed with Abdel-Hamied et al. (2016), and the ratio of the number of monocytes was 1.05 ± 0.022 in the infected cattle and in the control group as 1.07 ± 0.40 and explained that because monocytes are the host cells. The reason for the decrease in white blood cells and monocytes is believed to be the role of cytokines, in particular NTF- α (Forsyth et al. 1999). It is explained that the increase in monocyte cells in pathological conditions may be due to the proliferation of these cells during the course of the disease as a host of the infection defense mechanism (Col & Uslu 2007). The results of the study showed a decrease in the value of neutrophils, and this was agreed with Omer et al. (2002), and the reason for the decrease in neutrophils is due to the destruction of cells leukocytes by protozoan parasites accompanied by the formation of leukocytes due to the detrimental effect of toxic protozoan receptors (Hussein et al.

2007).

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