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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Cattle blood analyses for parasitic infestation in Mosul, Iraq

Al-Abadi B.H[1] and Al-Badrani B.A[2]

Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

Abstract

Microscopic examination of blood smears from 220 cattle of different regions in Mosul, Iraq revealed eight genus of parasites. Some of them were intraerythrocytic parasites like *Theileria spp*. (15.78%), *Babesia spp*. (10.0%), and *Anaplasma spp*. (8.0%). Other were *Mycoplasma wenyonii* (3.15%) and *Trypanosoma spp*. (6.48%), tachyzoite of *Toxoplasma gondii* (2.63%) and Microfilaria of *Setaria spp* (1.61%). *Ehrlichia spp*. (13.16%) were seen inside the cytoplasm of leukocytes. *Trypanosoma evansi* (23.07%), *T. theileri* (23.07%) and *T. uniform* (15.38%) were classified in this study according to the morphological characteristic and biometrical analysis. High parasitemia was recorded with *Hemoplasma* (50-100%) in comparison with other infections and most of these infections were in acute stage of the disease. Morphological changes of erythrocyte, leukocytes and platelets showed association with most of these blood parasites infections. Apoptosis in peripheral blood was detected during the acute *T. evansi* infection in cattle under light microscope. In the present study, we reported the blood parasites detected in cattle of Mosul, Iraq for the first report in Mosul, Iraq.

Keywords: Blood smear; blood parasites; cattle, Mosul, Iraq

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Introduction

Epidemiologic, clinical, immunodiagnostic and molecular techniques are used for diagnosis of diseases. Laboratory diagnosis is still based on morphological identification of the etiologic agent for most diseases. Films prepared from capillary or venous blood are of primary importance in diagnosing theileriosis, babesiosis, the acute stages of trypanosomiasis and microfilariasis. Blood smear analysis is commonly used to evaluate hematologic conditions, is infrequently used to diagnose infectious diseases. This is because of the rarity of diseases for which blood smear analysis is indicated. More information can be gained from examining the blood smear than from any other single hematologic procedure. **I**nterpretation of blood smears is commonly used to provide rapid laboratory evaluation of animals in veterinary emergency practice (Kroft, 2002). The microscopical examination of blood smear is considered one of the important methods for diagnosis of blood parasites in cattle. In some times, it represents the only step for final diagnosis of the infectious agents such as blood protozoa, rickettsia, trypanosome and microfilaria (Blevins et al., 2008).

The morphological changes of erythrocyte, leukocytes and platelets showed an association with most of these blood parasites infections. These changes may be specific or non-specific with disease. The nonspecific changes may include morphologic changes in leukocytes and erythrocytes (e.g. toxic granulations, macrocytosis) or identifying certain pathogens (like blood parasites) in a peripheral blood smear allows a rapid diagnosis (Allison and Meinkoth, 2010).

Theileria, *Babesia* and *Anaplasma* are considered to be the most important blood parasites of cattle in Mosul, Iraq (Hasoon, 1980; Goory; 1981; Shuker, 1989; Alsaad, 1990). Some outbreaks in exotic and crossbred cattle were mostly seen throughout the last years due to uncontrolled introduction of exotic breeds of cattle in Mosul city, Iraq in order to increase meat and milk production. This practice led to the

Corresponding author: Al-Abadi BH, Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq

introduction of new blood parasites which were not seen in Iraq previously such as *Trypanosomes* (Rhayma and AL-Badrani, 2012), *Mycoplasma wenyonii* (AL-Badrani and Rhayma, 2012), *Ehrlichia* (AL-Badrani, 2012). Therefore, this study was performed to diagnosis blood parasites in cattle in Mosul, Iraq.

Materials and Methods

In this study 220 cattle (native and exotic breeds), of which 130 calves (11-15 months) and 90 cows (2-5 years) were brought from different farms around Mosul city to the Veterinary Teaching Hospital, Veterinary Medicine University of Mosul, Iraq, with clinical signs of emaciation, fever, anorexia, lymph nodes enlargement, presence of ticks, anemia, hemoglobinurea and decreased milk production, edema of legs and teats of some cows.

Peripheral blood smears (two thin and two thick) were prepared from the ear vein of each animal. The smears were air dried, fixed in absolute methanol and stained with Romanowsky stains and examined under a light microscope using oil immersion (100x) for detection of any blood parasites by the methods described previously (Shuker, 1989; Alsaad, 1990). Positive results with *Mycoplasma wenyonii* infection were defined if one infected erythrocyte was found in 200 observed RBC (Yuan et al., 2009). Five hundred leukocytes were examined for *Ehrlichia* organisms, and the percentages of positive cells were calculated (Noaman and Shayan, 2009). The percentage of parasitemia of *Trypanosomes* (Anosa et al., 1992), microfilarias (Sharma and Joshi, 2002) and *Toxoplasma* tacyzoites (Petakov et al., 2002) in stained blood smears were estimated by calculating numbers in one hundred microscopic field under oil immersion (100x).

Wet smears were also prepared for studying erythrocyte morphology and detection of trypanosomes and microfilarias from their motility under 100x and 40x. A new methylene blue "wet mount" preparation was used for rapid information concerning the number of reticulocytes, platelets and Heinz bodies present. Identification of trypanosome species were based on their motility and using morphological differentiation on Giemsa or Leishman stained that showed trypomastigot which can be classified according to the biometrical data. For the biometrics characterization, 50 trypanosomes were measured using light microscope connected to a specific software (Image-prp Plus). The biometric data were compiled as described by Hoare (1972). Semi-quantitative evaluation of erythrocyte, leukocytes and platelets morphology based on average number of abnormal cells per 100x microscopic monolayer field (Weiss, 1984).

A total leukocyte number in blood smears (cells/µl) were estimated by determining the average number of leukocytes present per field and multiplying by 2000 at 100x (Metzger and Rebar, 2004). A differential leukocyte count was done by identifying 100 consecutive leukocytes using oil immersion. After the count was complete, the percentage of each leukocyte type present was calculated and multiplied by the total leukocyte count to get the absolute number of each cell type present per microliter of blood (Coles, 1986). Platelet numbers were also estimated in blood smear by multiplying the average number per field by 15,000 to get the approximate number of platelet/µl of blood (Webb et al., 2004; Rosenfeld and Dial, 2010).

Data were analyzed by SPSS statistical program and Means ± SD were determined for the studied parameters. One-way ANOVA was carried out to find the differences in the parameters. A value of the $P<0.05$ was considered significant in comparison of mean value of control group.

Results

Blood smear analysis revealed eight genera of blood parasites in 190 cattle. Some of them were intra-erythrocytic parasites like *Theileria* (15.78%), *Babesia* (10.0%), *Anaplasma* (8.0%). Others were epierythrocytic *Mycoplasma wenyonii* and *Haemobartonella bovis* (3.15%) and extra-erythrocytic parasites were included *Trypanosoma* spp. (6.48%), tachyzoites of *Toxoplasma gondii* (2.63%) and microfilaria of *Setaria spp*. (1.61%). Ehrlichia inclusions were also seen inside the cytoplasm of leukocytes in 13.16% of cattle in this study. The higher percentage of infection was recorded in the mixed infection (at least two to five parasites in the same blood smear of the same animal), it is about 42.63% (Table 1). The remaining 30 animals were free of infection.

Theileria annulata piroplasms (0.5-1.6 µm) were seen in blood smears of some cattle (Fig. 1). Morula stage of *Ehrlichia spp.* which appeared very clearly in the cytoplasm of neutrophil, lymphocyte and monocyte (Fig. 2 & 3). There are three species of *Babesia* recorded in blood smear of cattle, including *Babesia bovis* (1×1.5 µm) in 9 cases, *B. bigemina* (2×3 µm) in 6 cases and *B. divergens* (0.4×1.5 µm) in 4 cases (Fig. 4, 5 & 6). *Anaplasma marginale* appears as dense, rounded and deeply stained intraerythrocytic bodies, approximately 0.3–1.0 µm in diameter. Most of these bodies were located on or near the margin of the erythrocyte. This feature distinguished *A. marginale* from *A. centrale* (0.2 - 0.5 µm), as in the latter most of the organisms have more central location in the erythrocyte. However, particularly at low levels of rickettsemia, differentiation of these two species becomes difficult. The percentage of infected

erythrocytes varies with the stage and severity of the disease. Maximum parasitaemia in excess of 50% may occur with *A. marginale.* Multiple infections of individual erythrocytes are common during periods of high parasitaemiasosis (Fig. 7). Extraerythrocytic parasites like Hemoplasmes which include *Eperythrozoon wenyonii* (formerly *Mycoplasma wenyonii*) appeared on the surface of RBC and in the background of blood smear. *Haemobartonella bovis* appeared as a chain on the surface of RBC (Fig. 8).

This is the first report in Iraq of *Trypanosoma evansi, T. theileri and T. uniform* in cattle*. T. evansi* (17-30 μm) which was monomorphic, heavy motile in wet smear, with prominent undulating membrane of 3-5 folds (Fig. 9-A & B). *T. theileri* which was pleomorphic, large trypanosome (25-90 μm), the kinetoplast was rod shaped and its position was variable. The nucleus was in the anterior region of the body. The posterior end was attenuated. Numerous dividing forms were seen in blood film (Fig. 10). *T. uniform*, a small trypanosome (12-20 μm) with rounded posterior end. The kinetoplast was located near the posterior end of the parasite with free flagella and prominent undulating membrane (Fig. 11). This small *Trypanosome* was even smaller than most members of the Salivaria. Its average dimensions were closely similar to those of strains of *T. uniform* (Table 2). Microfilaria of *Setaria spp*. (155-290 μm) was recorded in 3 cases in thick smear and wet film (Fig. 12 &13). Tachyzoite of *Toxoplasma gondii* (5-6 ×2-3 μm) appeared as a crescent or banana shape having red nucleolus with blue cytoplasm after staining by Giemsa in 3 aborted cows and 2 calves (Fig. 14). High parasitemia was recorded with *Haemoplasmes* (*Mycoplasma wenyonii*) which was 50-100% in comparison with other infections and all infections were in acute stage of the disease (Table 3). Wright stain gave good results for morphological studies of blood cells, while Giemsa and Leishman stains gave the best results for identification of blood parasites especially *Theileria*, *Anaplasma* and *Mycoplasma.* Erythrocytic inclusions were detected in blood smears that stained with new methylen blue or brilliant cresyl blue, like basophilic stippling, nucleated erythrocyte, reticulocytosis, Heinz bodies, Howell jolly bodies especially in wet smear in cases associated with *Babesia* infection (Table 4). The presence of abnormal leukocyte morphology, such as toxic cytoplasm in neutrophils or increased reactive lymphocytes (more than 5%), appearance of Dőhle bodies, cytoplasmic vacuolation , hypersegmentation and nuclear indistinct are associated within most blood parasites infections, but more frequency were seen in *Trypanosoma* and *Theileria* infections (Table 5). In this study, we recorded the morphological changes in platelets with single and mixed infection of blood parasites (Table 6).

We also recorded a significant decrease in total leukocytic count with all blood parasites infections except within *Mycoplasma* and microfilaria of *Setaria spp.*, which increased in comparison with their numbers in control animals. Thrombocytopenia was recorded with all blood parasites in this study, but thrombocytosis was seen only within *Trypanosome* (Table 7). Neutropenia with lymphopenia were seen in all infection, except in Mycoplasma infection. Neutropenia with lymphocytosis and eosinophilia were recorded in blood smears of cattle infected with microfilaria of *Setaria spp.* in comparison with their numbers in the control group (Table 8).

Apoptosis was seen in peripheral blood smear of cattle with *Trypanosoma* infections (*T. evansi*) especially in neutrophils and lymphocytes. In infected

Table 1: number of cattle and percentage of infection with blood parasites

Blood parasites	No. calves	Percentage	No. cows	Percentage	Total numbers	Percentage
Mixed infection	49	44.54%	32	40.0%	81	42.63%%
Theileria	25	22.72%	5	6.25%	30	15.78%
Ehrlichia	3	2.72%	22	27.5%	25	13.16%
Babesia	10	9.09%	9	11.25%	19	10.0%
Trypanosoma	8	7.27%	5	6.25%	13	6.48%
Anaplasma	6	5.45%	2	2.50%	8	4.40%
Hemoplasmes	4	3.63%	2	2.50%	6	3.15%
Toxoplasma	3	2.72%	2	2.50%	5	2.63%
Microfilaria	2	1.81%	1	1.25%	3	1.61%
Total count	110	100%	80	100%	190	100%

Table 2: Measurements (μm) of *Trypanosoma spp*. in blood smear of cattle

Type of trypanosome	No. cases	Parasitemia(%)	PK	KN	PN	F	TL	N/NA	BW
(*T. theileri*)	3	23.07%±2.21	32.02±3.21	5.53±1.03	17.16±2.30	11.26±1.82	77.34±0.13	1.28±0.30	4.26±0.42
(*T. uniform*)	2	15.38%±2.16	0.86±0.07	3.16±1.35	3.02±1.16	4.75±1.20	17.42±2.61	0.53±0.08	2.82±0.52
(*T. evansi*)	3	23.07%±5.3	4.35±0.92	3.91±0.71	7.12±2.14	4.01±1.31	25.89±3.6	1.09±0.05	3.69±0.65

PK= Distance from the posterior end to Kinetoplast. KN= Distance from Kinetoplast to middle of nucleus. , PN= Distance from the posterior end to middle of nucleus. NA= Distance from nucleus to anterior extremity. F= Free flagellum, TL= Total length, including free flagellum

Table 3: percentage of parasitemia in blood smear of cattle which infected with blood parasites

Blood parasites	Percentage of parasitemia%	Blood parasites	Percentage of parasitemia
Theileria annulata	5-11%	*T. evansi*	3-15%
Ehrlichia spp.	5-10%	*Anaplasma marginale*	1.6–50%
Babesia bovis	1.6 – 2%	*Anaplasma centrale*	0.1-2%
Babesia bigemina	10 – 60%	*Mycoplasma wenyonii*	50-100%
Babesia divergens	3 – 4%	*Haemobartonella bovis*	0.1%
Trypanosoma theileri	1 – 5%	Microfilaria	1–4%
T. uniform	4 - 20%	*Toxoplasma gondii*	20-50%

Table 4: Semiquantative evaluation of erythrocyte inclusions in blood smear in cattle which infected with blood parasites

Erythrocyte inclusions	*Theileria*	*Ehrlichia*	*Babesia*	*Trypanosoma*	*Anaplasma*	*Mycoplasma*	*Toxoplasma*	Microfilaria
Reticulocyte	0	0	5-20%(2+)	5-20%(2+)	0	3-4%(1+)	0	0
Nucleate RBC	5-0(1+)	0	5-10(1+)	5-10(1+)	5-10(1+)	5-10(1+)	0	0
Basophilic stippling	0	0	11-25(4+)	6-10(2+)	0	1-5(1+)	0	1-5(1+)
Heinz bodies	3-5(1+)	3-5(1+)	3-5(1+)	3-5(1+)	3-5(1+)	3-5(1+)	0	0
Howell-jolly bodies	0	0	1-2(1+)	1-2(1+)	0	1-2(1+)	0	0
Hb.crystals	0	0	3-5(1+)	0	6-10(2+)	11-20(3+)	0	0

Table 5: The severity of degenerative changes in neutrophile and lymphocyte in cattle which infected with blood parasites

	Blood parasites in blood smear							
Type of degenerative changes in neutrophile and lymphocyte	*Theileria*	*Ehrlichia*	*Babesia*	*Trypanosoma*	*Anaplasma*	*Mycoplasma*	*Toxoplasma*	Microfilaria
Dőhle bodies	1+	1+	1+	1+	0	0	0	0
Cytoplasmic basophilia	0	0	0	0	0	0	0	0
Vacuolization		0	2+	0	0	0	0	0
Toxic granulation	3+	3+	0	0	0	0	0	0
Hypersegmentation	4+	0	0	4+	0	0	0	0
Granular lymphocyte	3+	0	0	0	0	0	0	0
Giant neutrophile	1+	0	0	0	1+	0	0	0
Neutrophilic inclusion	0	3+	0	0	0	0	0	0
Reactive lymphocyte	1+	1+	0	1+	0	0	0	0
Atypical lymphocyte	0	2+	0	0	2+	0	2+	0
Karyohexis/Pyknosis	0	0	0	1+	0	0	0	4+
Nuclear Indistinct	0	0	0	4+	0	4+	0	0

Table 6: Semiquantative evaluation in cattle which infected with blood parasites depending on the abnormal number of platelet /field under 100×

Morphological changes in platelet	*Theileria*	*Ehrlichia*	*Babesia*	*Trypanosoma*	*Anaplasma*	*Mycoplasma*	*Toxoplasma*	Microfilaria
Giant platelet	0	1-5 (1+)	0	6- 10(2+)	0	0	0	0
Elongated platelet	0	1-5 (1+)	0	1-5 (1+)	0	0	0	0
Adhesive platelet	0	30-200 (4+)	1-5(1+)	6- 10(2+)	11-25(3+)	6 – 0(2+)	1-5(1+)	6- 10(2+)
Activated platelet	0	1-5(1+)	1-5(1+)	6-10(2+)	0	0	1-5(1+)	1-5(1+)
Round platelet	0	0	0	1-5(1+)	0	0	0	0
Pseudopoda platelet	0	0	0	1-5(1+)	0	0	0	1-5(1+)

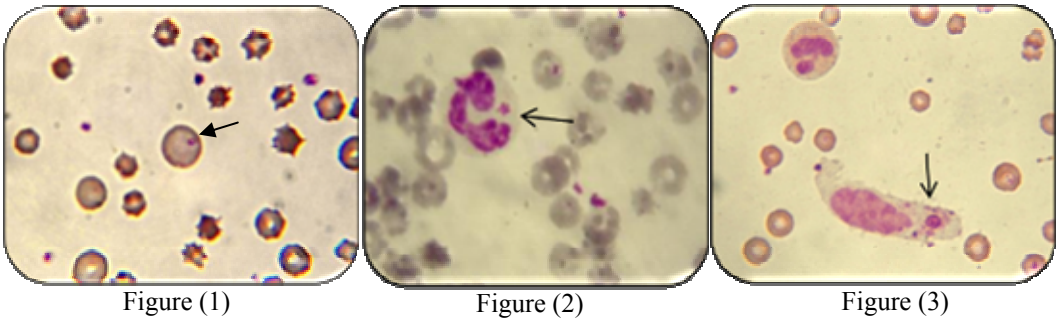


Figure (1) Figure (2) Figure (3)

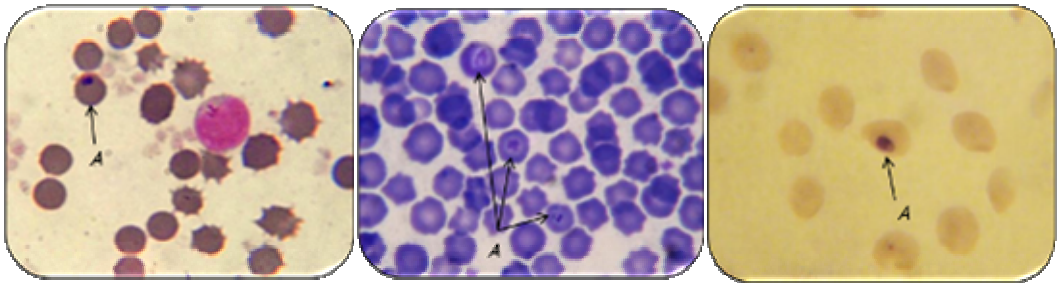


Figure (4) Figure (5) Figure (6)

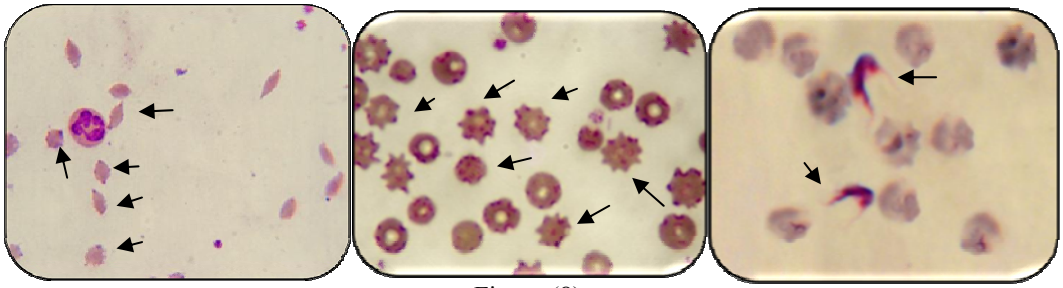


Figure (7) Figure (8) Figure (9-A)

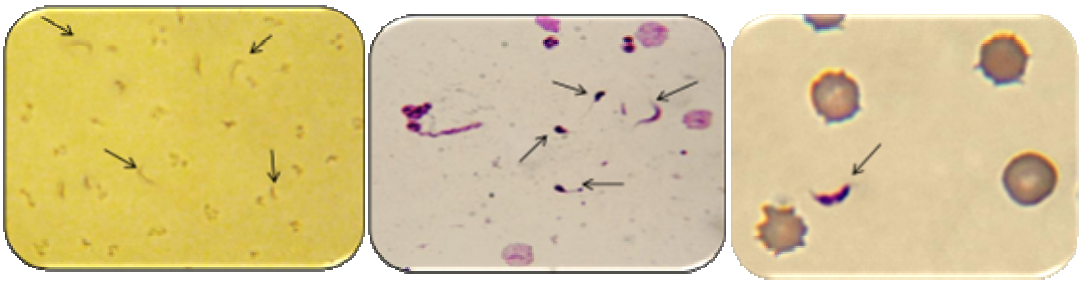
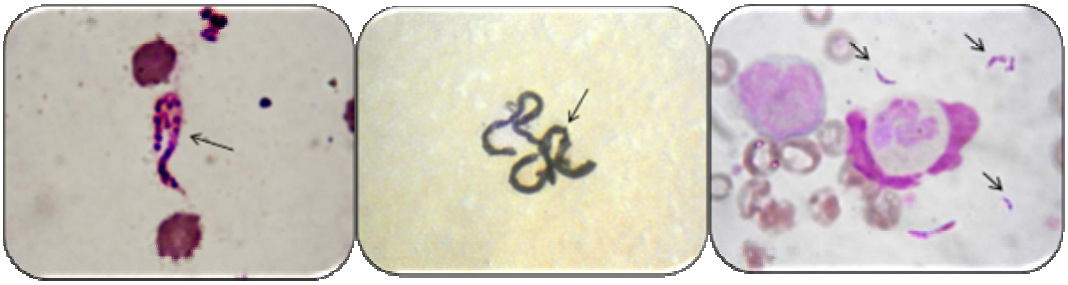


Figure (9-B) Figure (10) Figure (11)

Fig. 1: blood smear from a cow infected with *Theileria annulata*, Leishman stain, (1600x).
Fig. 2: blood smear from a cow infected with *Ehrlichia spp*, morula stage in cytoplasm of neutrophile, Leishman stain, (1000x).
Fig. 3: Morula stage in the cytoplasm of lymphocyte in calves, Giemsa stain (40x)
Fig. 4: Blood smear from a cow infected with *Babesia bovis* in cow, Giemsa stain (100x)
Fig. 5: Blood smear from a cow infected with Trophozoite of *B. bigemina*, Brillant cresyl blue (100x)
Fig. 6: Blood smear from a cow infected with *B. divergens*, Giemsa stain (100x)
Fig. 7: Multiple infections with *Anaplasma marginale* in Blood smear from a cow, Giemsa stain (100 x)
Fig. 8: High parasitemia of *Mycoplasma wenyonii* in Blood smear from a cow, Giemsa stain (100x)
Fig. (9-A): *Trypanosoma evansi* in Blood smear from a cow, Giemsa stain (100x)
Fig. (9-B): *Trypanosoma evansi* in unstained wet film from a cow (40x)
Fig. 10: *T. theileria* in blood smear from a cow, Leishman stain (100x)
Fig. 11: *T. uniform* in blood smear from a calve, Leishman stain (100x)



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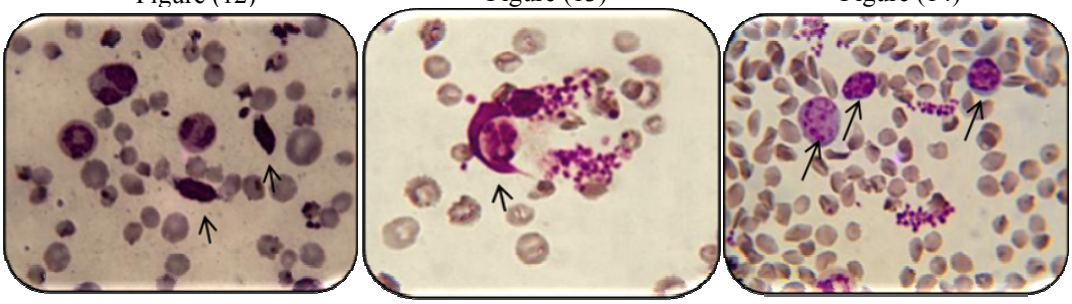

Figure (15) Figure (16) Figure (17)

Fig. 12: Microfilaria of Setaria in blood smear from a cow, Leishman stain (40x)
Fig. 13: Thick blood film from a cow infected with Microfilaria (40x)
Fig. 14: *Toxoplasma gondii* in blood smear from cow, Leishman stain (100x)
Fig. 15: Elongated lymphocyte with condense chromatin in cow infected with *T.evansi*, Leishman stain (100x)
Fig. 16: Karyolysis of neutrophile in blood film from cow infected with *T.evansi*, Leishman stain (100x)
Fig. 17: Perinucleur and condensed chromatin in neutrophile of blood film from cow infected with *T. evansi*, Leishman stain (100x)

Table 7: Total counts of leukocyte and platelets in animals infected with blood parasites

	Blood parasites								
Variable	Control	*Theileria*	*Ehrlichia*	*Babesia*	*Trypanosoma*	*Anaplasma*	*Mycoplasma*	*Toxoplasma*	Microfilaria
Leukocyte count x 10^3 µl	9.63± 0.91	2.3± 0.21	4.80± 0.73*	2.8± 0.42*	3.0± 0.61*	2.0± 0.39*	11.4± 1.72*	3.46± 0.59*	11.38± 1.32*
Platelets x 10^3/µl	340.2± 23.60*	181± 15.90*	196.7± 15.20*	183.9± 19.30*	532*± 25.20*	150± 12.40*	337± 13.40*	123.3± 10.32*	186.27± 18.49*

Values are mean ± SD; *Mean significant at P≤0.05

Table 8: Absolute numbers of differential leukocyte count (cell/ µl) in cattle infected with blood parasites

Types of leukocyte	Control	*Theileria*	*Ehrlichia*	*Babesia*	*Trypanosoma*	*Anaplasma*	*Mycoplasma*	*Toxoplasma*	Microfilaria
Neutrophile	3370± 25.26*	1100± 30.21*	1090± 13.90*	1160± 24.90*	2250± 13.7*	1179± 23.4*	5990± 30.6*	1700*± 21.0*	1812± 32.2*
Lymphocyte	5811± 29.01*	1090± 23.14*	3233± 17.34*	1530± 36.0*	4750± 53.0*	1080± 24.0*	2430± 25.60*	2300± 31.00*	8200± 53.6*
Monocyte	118.0± 10.22*	330.0± 43.61*	411.4± 33.91*	200.0± 16.6*	555.0± 19.0*	113± 30.0*	830.0± 17.07*	230.0± 11.2*	307.8± 52.0*
Eosinophil	340.0± 10.09*	300.0± 20.91*	200.0± 12.01*	600.0± 10.0*	78.01± 11.01*	600.0± 11.1*	151.0± 202*	460. 9± 50.0*	840± 267*
Basophile	0	0	0	0	0	0	0	0	0

Values are mean ± SD; *Mean significant at p≤ 0.05

animals, monocytes with phagocytosed red blood cell were also seen. A few lymphocytes (2–3% of normal lymphocytes) revealed shrinkage of the cytoplasm with condensed nuclear chromatin, nucleules fragmentation, and cytoplasmic blebbing. No apoptotic cells were observed in all blood smears made from the non-infected control cattle. Other light microscopy findings revealed a few bi-nucleated lymphocytes; lymphocytes with azurophilic granules and reactive lymphocytes (Fig. 15, 16 & 17).

Discussion

Blood smear analysis is especially useful for diagnosis of eight infectious diseases: theileriosis, ehrlichiosis, babesiosis, anaplasmosis, trypanosomiasis, toxoplasmosis and filariasis in cattle. Thick smears are especially sensitive, but thin smears are useful for identifying the species and for estimating the level of parasitemia. Thick blood films as used for the diagnosis of babesiosis are not appropriate for the diagnosis of Anaplasma. Thick blood films are 10 times more sensitive than thin blood films and are therefore very useful for the detection of low level *Babesia spp*. infections (de Vos et al., 2004). Species differentiation is good in thin films but poor in thick films. Thin films are usually adequate for detection of acute infections, but not for detection of carriers where the parasitemias are mostly very low (Blevins et al., 2008).

In this study, the mixed infection (42.63%) with blood parasites was recorded. A signs of regenerative anemia like anisocytosis, polychromacia and basophilic stippling are associated with acute theileriosis, hemoplasmosis, babesiosis, trypanosomosis and ehrlichiosis. Thus, indeed the peripheral blood smear evaluation will be necessary to understand the severity and stage of the disease (Issi et al., 2010).

Acute anaplasmosis can be differentiated from chronic cases by the appearance of signs of regenerative anemia and presence of spherocytosis and erythrophagocytosis of infected and non-infected erythrocytes, which is useful in diagnosis of anaplasmosis (Bock et al., 2004).

A positive finding of *B. bovis* in blood smears is usually reported as being significant regardless of the parasitaemia (Böse et al., 1995). With *B. bigemina*, interpretation of the results is often difficult because this parasite is sometimes found in clinically healthy animals. A report of a significant *B. bigemina* infection is based on 1% or more of red blood cells being infected. A parasitaemia of less than 1% may be regarded significant where the infection is accompanied by marked anemic changes of the cells (Reticulocytosis and presence of Heinz bodies) and the history is strongly suggestive of tick fever (de Vos et al., 2004). Results of examination of smears for *A. marginale* are also difficult to interpret. A positive result is usually reported as being significant if more than 5% of red blood cells are infected, and less significant if associated with signs of regenerative anemia (Potgieter and Stoltsz, 2004). Intercurrent or concurrent conditions play an important role in this study like Ehrlichia and Hemoplasma. It is expected that these concurrent infections in animals are associated with imported animals from the neighbouring countries such as Turkey, Iran and Syria. Diagnosis of bovine ehrlichiosis was based on demonstration of *Ehrlichia* morula in leukocytes. Neutrophils were predominantly infected during the acute phase of the disease, whereas monocytes were infected towards the end (Noman and Shayan, 2009). Neutrophils and eosinophils are the cells in which the organisms primarily multiply, whereas monocytes and lymphocytes are secondary host cells for *E. phagocytophila* during the acute phase of the disease (Aktase et al., 2010). Leukopenia has been attributed to possible suppression of bone marrow production or to the destruction of infected cells (Pusterla et al., 1997). We assumed that the thrombocytopenia was due to an increased consumption of platelets and premature destruction in the spleen, as occurs in infections with other *Ehrlichia* species. Immunological and inflammatory processes play an important role in platelet consumption (Smith et al., 1975). Eperyrethrozon *wenyonii* (*Mycoplasma wenyonii*) and *Haembarotenella bovis* in cattle were reported for the first time in Mosul, Iraq by AL-Badrani and Rhayma (2012), *Ehrlichia spp.* (AL-Badrani, 2012). Differentiation of *Epeythrozoon* versus *Haemobartonella* is some arbitrary, but *Haemobartonella* does not usually occur as a ring form and it is not present free in the plasma, while *Eperythrozoon* does occur as a ring form and it can be found free in the plasma (Weiss and Moulder, 1984). Several factors are responsible for causing anemia due to *T. evansi* infection, the production of haemolysin by trypanosomes resulting into haemolysis of RBCs, extravascular destruction of RBCs, the erythrophagocytosis, immune mediated depression of erythropoiesis and non-specific factors, which increase red cell fragility, may be responsible for anaemia (Laha and Sasmal, 2007).

Careful examination of peripheral blood smears in cattle suspected of toxoplasmosis may be warranted. Tachyzoites play an important role in pathogenesis during acute toxoplasmosis (Desmonts and Couvreur, 1974; Roberts and McLeod, 1999). Studies on the migratory characteristics of Toxoplasma tachyzoites are directly relevant to the role of this stage in dissemination-related acute pathology and in reactivation of chronic infections. Ingested parasites (oocysts and bradyzoites in tissue cysts) invade the

intestine and differentiate into tachyzoites followed by spread of the organism hematogenously and via lymphatics and upon reactivation of chronic infections in humans (Dubey, 1997). The ability to rapidly cross epithelial barriers and reach the circulation may be an important component of dissemination *in vivo*, particularly to sites of immune privilege, e.g., the central nervous system and the developing fetus (Reiter-Owona et al., 2000). In peripheral blood, *T. gondii* infection caused a significant decrease in the total number of white blood cells and platelets. Petakov et al. (2002) showed that acute infection by *Toxoplasma gondii* in mice was associated with leucopenia, thrombocytopenia and bone marrow hypoplasia, while, in spite of the infection-induced damage of the granulocyte cell lineage, in bone marrow stimulated production of granulocytes was revealed. Lymphocytosis and eosinophilia were recorded with microfilaria of *Setaria spp*. in blood film which is associated with chronic parasitism. The degree of lymphocytosis occurred depend upon the intensity of parasitemia (Sharma and Joshi, 2002).

Trypanosome-induced peripheral blood cell apoptosis has been documented as a feature of trypanosomiasis by Happi et al. (2012). In most parasitic disease conditions, blood cell apoptosis is very rare to find by light microscopy examination as they are rapidly engulfed by adjacent cells such as monocytes (Savill and Fadok, 2000). Peripheral blood leukocyte apoptosis was more readily noticeable in *T. bucei* infected rats that survived longer. This may suggest an increase in apoptotic cells or a defect in their clearance (Happi et al., 2012). While an increase in apoptotic cells is most probable, it was observed that during that same period there was an increase in the main blood phagocytic cells (monocytes) and the reduction in the leukocyte count.

In conclusion, blood smear analyses are still a first step in the laboratory diagnosis of blood parasites in animals. The information about the occurrence of these pathogens may provide basis for further research about cattle blood parasites in Iraqi environmental conditions.

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