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Study of Clinical, Epidemiological and Heamatological changes of Heamoparasites infection in Cattle of Diyala Province-Iraq.

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ABSTRACT

Haemoparasites of animals reduce livestock productivity and could lead to high mortality. This study aims at determining the prevalence, clinical and heamatological change of haemoparasites in cattle of different district in Diyala province. A total of two hundred blood samples were collected from different age, breed and sex of cattle, from 2014 to 2015. The blood samples were examined for haemoparasites by making thin blood smear and staining with Giemsa's stain. Over all prevalence rate infection of haemoparasites showed that (100%), (90%), (80%) and (56%) for summer, spring, autumn and winter seasons of 2014 and (100%), (100%), (92%) and (68%) for summer, spring, autumn and winter seasons of 2015 respectively were recorded as positive for blood parasites using thin blood film. *Babesia bigemina*, *B. bovis*, *Theileria annulata* and *Anaplasma marginale* able to produce the infection in all ages levels and the highest percentage of infection in age groups less than one year old (92.8%), while the lower prevalence of infection occurs in cattle over than 3-5 years old (80.0%) with significant differences $P < 0.05$. The study showed high prevalence of infection in cross breed (91.1%) compared with local breeds (82.7%) and effect of sex of animals show high prevalence in female (88.23%) than male (84.69%) with significant differences $P < 0.05$. The hematological analysis showed statistically a significant decreases was observed in total erythrocyte count (TEC), mean hemoglobin concentration (Hb), packet cell volume (PCV), and slight increase of total leukocyte count (TLC) ($P < 0.05$), whenever, variable difference in differential white blood cells compared with normal value significantly ($P < 0.05$) blood feature complained by macrocytic hypochromic anemia with abnormal morphology of erythrocyte as anisocytosis, poikilocytosis, basophilic stippling, hypochromasia, cremated RBC and polychromasia. This study is the first report in Diyala province.

Keywords: epidemiology, heamatology, heamoparasites, cattle

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Introduction

Haemoparasites have generally been shown to cause destruction of red blood cells resulting in anaemia, jaundice, anorexia, weight loss and infertility [1]. Parasitic diseases have debilitating impact on human and animal health worldwide particularly in developing countries [2]. The direct losses caused by the parasites are attributed to acute illness and death, premature slaughter and rejection of some body parts at meat inspection. Indirect losses include the reduction of productive potential such as decreased growth rate, weight loss in young growing animal and late maturity of slaughter stock [3]. Farmers may not appreciate the effects of these haemoparasites on their animals, perhaps due to the subclinical nature of presentation and chronic nature on the affected animals [4]. *Babesia*, *Theileria* and *Anaplasma*, are Tick-borne blood parasites which globally impact on animal health and economic.

Babesiosis is a disease caused by parasitic protozoa of the genus *Babesia*. Symptoms in animals are anorexia, fever, anemia, increased respiratory rate and pulse rate, jaundice, diarrhea, constipation, hemoglobinuria and respiratory problems. Fever that occurs can lead to abortion and decreased sperm fertility [5].

Theileriosis is a disease caused by protozoa of the genus *Theileria*. It is an obligate intracellular parasite. Clinical symptoms that occur are fever, anorexia, enlargement of prescapular lymph node, decreased milk production and weight, respiratory problems, poor growth and production, jaundice, anemia and abortion [6].

Anaplasmosis in cattle caused by obligate intra-erythrocytic parasite of the genus *Anaplasma*. Symptoms that occur are fever that last for 4 to 10 days, anorexia, weight loss, lethargy, cough and increased respiratory rate and pulse rate, abortion, decreased milk production and quality of cement [7].

The aim of this study is to investigate the presence of anaplasmosis, babesiosis, theileriosis in cattle of Diyala province and the influence of season period on infections.

MATERIALS AND METHODS

Study area

The area of the study includes four locations in the Diyala province these locations are Al Khalis, Al Mughdadia, Baqubah and Baldruze

Sample collection

Blood were collected from 200 Cattle of both sexes and different ages were examined clinically respiratory and heart rate, body temperature, mucus membrane and percentage of dehydration from 2014 for 2015. About 5ml of blood were collected from the severed jugular vein of each animal into ethylene diaminetetra acetic acid (EDTA) bottle and the samples were transported to the Veterinary Clinical Pathology Laboratory of the Faculty of Veterinary Medicine, University of Diyala for parasitological and haematological analyses. On the other hand lymph samples were collected from the enlargement superficial lymph node specially prescapular lymph node by puncture and diatheses the lymph to marking lymph film according the procedure of [8].

Sample preparation

A drop of blood was placed on a grease free glass slide and a thin blood smear was prepared from each blood sample, air-dried, fixed in methanol for 2-3 minutes, stained in 10% Giemsa's stain and rinsed in buffered water according [9]. The smears were examined at X100 magnification (oil immersion) on an Olympus Microscope. Blood sample was introduced into a plain glass microhaematocrit tube, one end of the tube was sealed using plasticine and the tubes were spun for 5 min at 1500 rev in a microhaematocrit centrifuge. Packed Cell Volume (PCV) was determined using a haematocrit reader and Total White Blood Cell count (TWBC) was estimated using the Neubauer Counting Chamber [9]. And DLC was performed by manual method as per [10].

RESULTS

The clinical signs of bovine theileriosis cause by *Theileria annulata* characterized by fever 40.5°C , anorexia, enlargement of superficial lymph nodes, nasal and ocular discharges and salivation is the most common signs. Yellowish soft diarrhea in small age groups and constipation is recorded insome cases. Increase of respiratory rate and heart rate , oedema and icterus and tick infestation.

The clinical signs recorded in some cases of bovine babesiosis characterized by high fever (40.4 to 41°C), anorexia, depression, weakness, and a fall in milk yield in dairy cows. Hemoglobinuria and the color of urine is dark-red to brown. On the other hand Respiratory and heart rates are increased, and paleness of mucus membrane and tick infestation. Whenever, clinical signs recorded in some cases of bovine anaplasmosis passing from pyrexia, paleness of mucus membrane, jaundice, tick infestation, depression, weakness, a fall in milk yield , high fever, and labored breathing in severe cases.

The morphological examination of thin blood smear showed that the Babesia in bovine B. bigemina blood smears were identified as large, pleomorphic piroplasms and B. bovis small piroplasms aligned at obtuse angle. Anaplasma species was identified as *A. marginale*. While, the Theileria species were identified as *T. annulata* (piroplasms including cocci, rod, comma, signet-ring, and pear-shaped forms with abnormalities in erythrocyte structure and observe the schizont stage Koch's blue body figures (1,2)

The presence of blood parasites in cattle in Diyala province was investigated during 2014 and 2015 years and different seasons. Over all prevalence rate infection of heamoparasites showed that (100 %), (90 %), (80%) and (56%) for summer, spring, autumn and winter seasons of 2014 and (100%) , (100%), (92 %) and (68%) for summer, spring, autumn and winter seasons of 2015 respectively were recorded as positive for blood parasites using thin blood film. All results are summarized in table (1).

Table (1) showed the prevalence of *Theileria annulata* infestation was(100%), (100%),(83.3%) and (60.0%)in Summer, Spring, autumn, and winter of 2014 whenever was (100%), (100%), (90.9%) and (69.2%) in Summer, Spring, autumn, and winter season of 2015 respectively. On the other hand the prevalence infestation of *Babesia* species infestation was (100%), (100%), (71.4%) and (62.5%) in Summer, Spring, autumn, and winter of 2014 whenever was (100%), (100%), (100%) and (66.6%) in Summer, Spring, autumn, and winter season of 2015 respectively. Unfortunately, the prevalence of *Anaplasma* species infestation was(100%), (83.3%), (83.3%) and (42.8%) in 2014 and (100%), (100%), (75.0%) and (66.6%) in Summer, Spring, autumn, and winter season of 2015 respectively.

The result of epidemiological study were showed a high percentage to infection was occurs in Baldruze90 % Al Kalis(88.8%) ,Al Mughdadia (83.3%) % and Baqubah (84.4%) with significant differed in $P>0.05$ table (2) and figure (3).

The present study showed that *Babesia bigemina*, *B. bovis*, *Theileria annulata* and *Anaplasma marginale* able to produce the infection in all ages levels and dependent on blood film examination registered the highest percentage of infection in animals with age group less than one year old (92.8%), while the lower percentage of infection occurs in animals over than 3-5 years old of infection (80%) with significant differences $P<0.05$ table (3).

As shown in table (4) based on blood film examination, the percentage of babesiosis , theileriosis and anaplasmosis were widely affects with cattle breeds and the study registers high percentage of infection in cross breed (91.1%) compared with local breeds (82.7%) with a significant differences $P<0.05$.

The results of prevalence study show effect of heamoparasites infection on sex of animals with significant difference $P>0.05$ compared with females(88.23%)than male (84.69%) as in table (5).

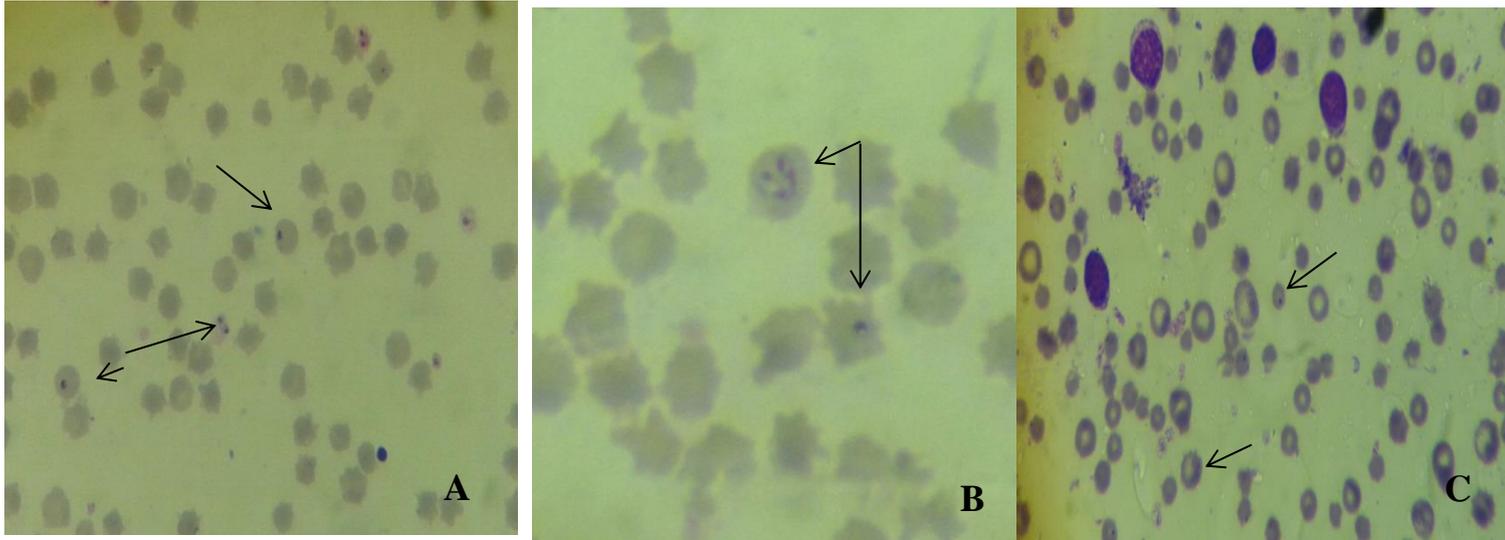


Fig.(1) A- Shows the erythrocyte stage of *Theileria annulata* infection arrow (Giemsa stain ; oil immersion, 1000X).

B- Shows the erythrocyte stage of *Babesia bigemina* infection (arrow) (Giemsa stain ; oil immersion, 1000X).

C- Shows the erythrocyte stage of *Anaplasma marginale* infection, arrow (Giemsa stain ; oil immersion, 1000X).

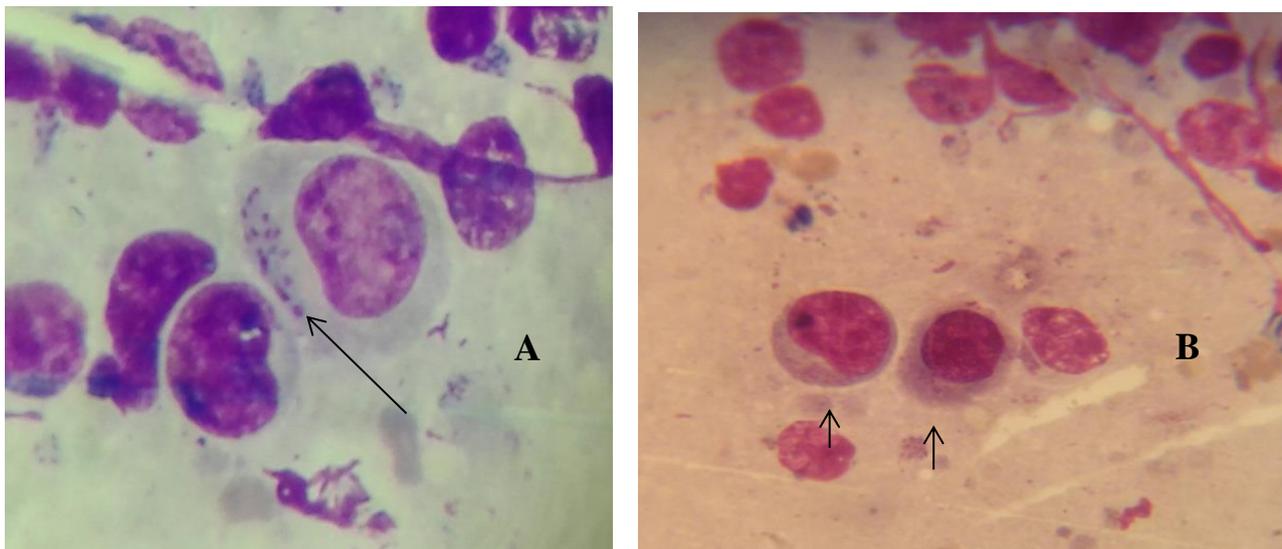


Fig.(2) A- lymph smear of enlarged prescapular lymph node show macroshizonts of *Theileria annulata* inside lymphocyte of infected cattle, arrow (Giemsa stain ; oil immersion, 1000X)

B- lymph smear of enlarged prescapular lymph node show microshizonts of *Theileria annulata* in lymphocytes of infected cattle, arrow (Giemsa stain ; oil immersion, 1000X)

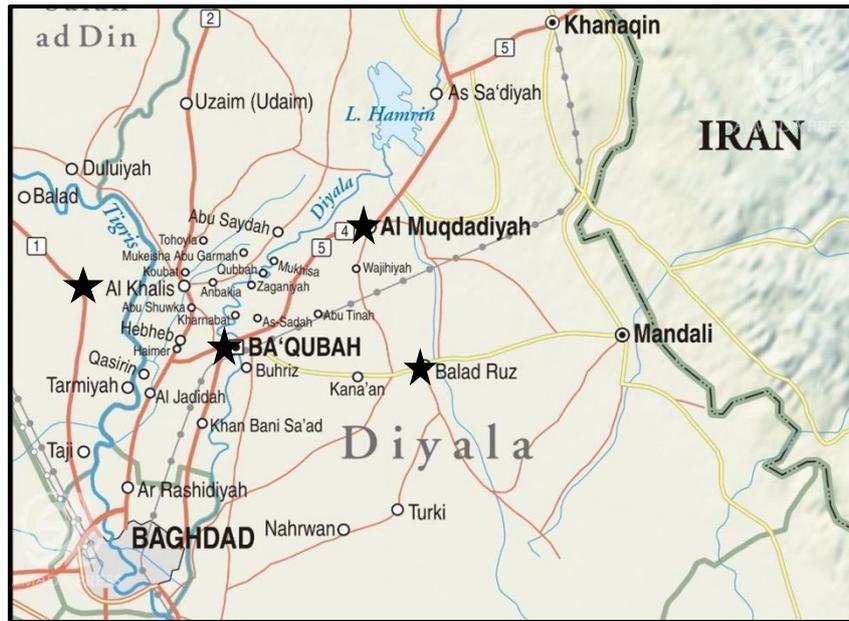


Figure 3 - Area of study under investigation for heamoparasites infection in cattle (★ : areas of samples collection) :Google, E (2015). Diyala city . <http://www.google.iq/maps/>. Diyala City, www.google.com [29].

Table (1) Prevalence rate and seasonal distribution of *Theileria spp*, *Babesia spp* and *Anaplasma Spp*.

Years	Season	N.E	N.P	Prevalence(%)						Over all prevalence (%)
				<i>Theileria spp</i>		<i>Babesia spp</i>		<i>Anaplasma Spp</i>		
				N.E	N.P	N.E	N.P	N.E	N.P	
				(%)	(%)	(%)	(%)	(%)	(%)	
2014	Winter (Dec.-Jan.-Feb.)	25	14	10	6	8	5	7	3	(56%)
				(60.0%)		(62.5%)		(42.8%)		
	Spring (Mar.-Apr.-May)	25	24	12	12	7	7	6	5	(90%)
				(100 %)		(100 %)		(83.3%)		
	Summer (Jun. Jul.-Aug.)	25	25	14	14	6	6	5	5	(100%)
				(100 %)		(100%)		(100%)		
	Autumn (Sep.-Oct.-Nov.)	25	20	12	10	7	5	6	5	(80%)
				(83.3%)		(71.4%)		(83.3%)		
		100	83	48	42	28	23	24	18	(83%)
				(87.5%)		(82.1%)		(75.0%)		
2015	Winter (Dec.-Jan.-Feb.)	25	17	13	9	9	6	3	2	(68%)
				(69.2%)		(66.6%)		(66.6%)		
	Spring (Mar.-Apr.-May)	25	25	15	15	5	5	5	5	(100%)
				(100 %)		(100 %)		(100 %)		
	Summer (Jun. Jul.-Aug.)	25	25	13	13	8	8	4	4	(100%)
				(100 %)		(100 %)		(100 %)		
	Autumn (Sep.-Oct.-Nov.)	25	23	11	10	10	10	4	3	(92%)
				(90.9%)		(100%)		(75.0%)		
		100	90	52	47	32	29	16	14	(90%)
				(90.4%)		(90.6%)		(87.5%)		
Total		200	173	100	89	60	52	40	32	(86.5%)
				(89.0%)		(86.7%)		(80.0%)		

$\chi^2=8.625$ & $P= 0.0134$ S.D

N.E = Number of examined animals , N.P = Number of positive infected, $\chi^2=$ Chi- Square , P = probability value S.D Significant difference

Table (2) Relation between the area of study and prevalence rate of theileriosis , babesiosis and anaplasmosis infection.

Area of study	N.E	N.P (%)	Prevalence rate (%)			X ² & P
			<i>Theileria spp</i> N.P (%)	<i>Babesia spp</i> N.P (%)	<i>Anaplasma Spp</i> N.P (%)	
Baldruze	50	45 (90%)	27(60.0%)	12 (26.7%)	6 (13.3%)	1.359 & 0.506 S.D
Al Khalis	45	40 (88.8%)	20(50.0%)	11(27.5%)	9(22.5%)	1.359 & 0.506 S.D
Al Muqdadia	60	50 (83.3%)	24 (48.0%)	16(32.0%)	10(20.0%)	2.356&0.307
Baqubah	45	38 (84.4%)	18(47.4%)	13(34.2%)	7(18.4%)	2.05& 0.358 S.D
Total	200	173	89(51.4%)	52 (30.0%)	32(18.5%)	8.625& 0.0134 S.D

Table (3):Relation of age of animals and prevalence of theileriosis , babesiosis and anaplasmosis infection.

Age of animals	N.E	N.P (%)	Prevalence rate (%)			X ² & P P<0.05
			<i>Theileria spp</i> N.P (%)	<i>Babesia spp</i> N.P (%)	<i>Anaplasma Spp</i> N.P (%)	
2 -12 months	42	39 (92.8%)	27(69.2%)	8(20.5%)	4(10.3%)	8.496& 0.014 S.D
1 year to 2 years	60	55 (91.6%)	34 (61.8%)	15(27.3%)	6(10.9%)	4.545& 0.103 S.D
2 years to 3	48	39 (81.2%)	17(43.6%)	15(38.5%)	7(17.9%)	0.903&0.636 S.D
4 years and more than 5 years	50	40 (80%)	11 (27.5%)	14(35.0%)	15(37.5%)	1.193&0.550 S.D
Total	200	173	89 (51.4%)	52 (30.0%)	32 (18.5%)	8.625 & 0.0134 S.D P<0.05

Table(4): Breed susceptibility of theileriosis , babesiosis and anaplasmosis in cattle of Diyala province.

Breed	N.E	N.P (%)	Prevalence rate (%)			X ² & P
			<i>Theileria spp</i> N.P (%)	<i>Babesia spp</i> N.P (%)	<i>Anaplasma Spp</i> N.P (%)	
Native breed	110	91(82.7%)	46(50.5%)	24 (26.4%)	21(23.1%)	1.978&0.371 S.D
Crossbreed	90	82(91.1%)	43 (52.4%)	28(34.1%)	11 (13.4%)	7.134&0.028 S.D
Total	200	173	89 (51.4%)	52 (30.0%)	32 (18.5%)	8.625 & 0.0134 S.D

Table(5): Relation of sex animals and prevalence of theileriosis , babesiosis and anaplasmosis infection.

Sex	N.E	N.P (%)	Prevalence rate (%)			X ² & P
			<i>Theileria spp</i> N.P (%)	<i>Babesia spp</i> N.P (%)	<i>Anaplasma Spp</i> N.P (%)	
Male	98	83(84.69%)	42(50.6%)	22(26.5%)	19(22.9%)	2.926&0.231 S.D
Female	102	90(88.23%)	47(52.2%)	30(33.3%)	13(14.4%)	5.429&0.066 S.D
Total	200	173	89 (51.4%)	52 (30.0%)	32 (18.5%)	8.625 & 0.0134 S.D P<0.05

The hematological analysis showed statistically a significant decreases was observed in total erythrocyte count (TEC), mean hemoglobin concentration (Hb), packed cell volume (PCV), and slight increase of total leukocyte count (TLC) ($P < 0.05$), whenever, variable difference in differential white blood cells compared with normal value significantly ($P < 0.05$) as in table (6). On the other hand the animals infected by bovine theileriosis, babesiosis and anaplasmosis complained of macrocytic hypochromic anemia with abnormal morphology of erythrocyte as anisocytosis, poikilocytosis, basophilic stippling, hypochromasia, cremated RBC and polychromasia as in fig.(1:A,B,C).

Table (6): Hematological parameter in animals of study infected by theileriosis, babesiosis and anaplasmosis.

Blood Parasites	Blood parameters of infected animals									
	TEC/ μ l M \pm SD	PCV% M \pm SD	Hb g/dl M \pm SD	TLC/ μ l M \pm SD	B. Neu. M \pm SD	Seg. Neu. M \pm SD	Baso. M \pm SD	Eosino. M \pm SD	Mon. M \pm SD	Lymph. M \pm SD
<i>T.annulata</i>	3721400 \pm 716854.1 SD*	22.6 \pm 4.22 *SD	6.8 \pm 1.3 *SD	1047 \pm 4695.7 **SD	215.1 \pm 217.3 **SD	4344.8 \pm 1815.1 **SD	54.6 \pm 83.3	1133.04 \pm 415.8 **SD	1113.02 \pm 433.9 *SD	3808.3 \pm 1877.5 **SD
<i>B.bigemina</i> <i>B.bovis</i>	2879870 \pm 2130185.9 SD*	24.5 \pm 4.89*SD	7.2 \pm 1.1* SD	6722.5 \pm 525.92 *SD	81.18 \pm 88.61	2575.8 \pm 311.5	0.0 \pm 0. 0	738.9 \pm 87.22 **SD	778.8 \pm 108.2 *SD	2462.6 \pm 477.7
<i>A.marginale</i>	3974000 \pm 795694.7 *SD	24.2 \pm 5.67 *SD	6.5 \pm 0.5 *SD	7562 \pm 455.71 *SD	76.8 \pm 78.43	2597.4 \pm 211.7	29.4 \pm 40.4	742.4 \pm 119.1 **SD	995.9 \pm 89.7 *SD	2880.3 \pm 263.1 *SD
Non infected	Blood parameters of healthy animals									
	5998500 \pm 671104	34.81 \pm 1.40	10.9 \pm 1 .03	8537.4 \pm 519.1	85.3 \pm 1.3	3158.8 \pm 3.76	0.0 \pm 0. 0	682.9 \pm 1. 5	768.4 \pm 1.1	3841.8 \pm 4. 58
TEC (Total erythrocyte count); PCV(Packed cell volume); Hb(Hemoglobin concentration); TLC(Total leukocyte count); B. Neu.(Band neutrophils); Seg. Neu.(Segmented neutrophils); Baso.(Basophils); Eosino.(Eosinophil); Mon.(Monocytes); Lymph.(Lymphocyte); M \pm SD(Mean \pm Significant difference) *SD (decrease with Significant difference), **SD (increase with Significant difference) at $P < 0.05$										

DISCUSSION

Theileria, Babesia and Anaplasma, are tick-borne blood parasites which globally impact on animal health and economic. In this study the diagnosis of *Babesia bigemina* and *Babesia bovis*, *Theileria annulata* and *Anaplasma marginale* in blood smear of cattle, was made in Diyala Province. It is noteworthy that there was no previous reports for the occurrence of these blood parasites in native and cross breeds of animals in eastern part of Iraq. Previous study by [11] in Baghdad and [12,13] in Basrah reported *Theileria annulata* in cattle.

Based on morphological characteristics the *Babesia* in bovine *B. bigemina* blood smears were identified as large, pleomorphic piroplasms and *B. bovis* small piroplasms aligned at obtuse angle [14] while, the *Theileria* species were identified as *T. annulata* (piroplasms including cocci, rod, comma, signet-ring, and pear-shaped forms with abnormalities in erythrocyte structure and observe the schizont stage koch's blue body [12,15]. *Anaplasma* species was identified as *A. marginale* [16].

In general the pyrexia occurs due to the liberation of endogenous pyrogens because cellular lysis and high level of parasitemia lead to the stimulation of thermoregulatory center in the hypothalamus [13,17].

Unfortunately, anorexia could be attributed to present fever [18]. The enlargement of superficial lymph node in bovine theileriosis could be explained by lymphoid hyperplasia in early stage of disease that occurs due to increases of proliferation of microschorizonts inside the lymphocyte caused inflammatory reaction in the infected lymph node [12,13,19].

Respiratory signs occur due to the accumulation of edematous fluid inside the lung and thoracic cavity [17]. The jaundice is due to the destruction of the blood cells and their contents being released into the blood stream.

The prevalence of theileriosis is higher when compared with babesiosis and anaplasmosis in this study this agreement with other authors [19,20,21]. The prevalence of theileriosis, babesiosis and anaplasmosis were higher in hot and humid season (summer, spring, and autumn) which related to the number of ticks and insects in hemoparasites transmission, most new disease cases are seen in late spring and early summer. this agreement with [23,24]. Whenever, the prevalence of infection were lower in cold season (winter) which related to cattle that recovered from blood parasites infection would be a carrier because it can transmit the disease from blood either through biological or mechanical transmission this closed agreed with [25].

The high prevalence of bovine theileriosis and babesiosis infection in small age groups (2 -12 months) and lower percentage of infection large age groups (4 years and more than 5 years) this result agreement with [11,12,13,20,21]. These results are explained by the small age groups being more susceptible to infection because does not keep immunity against the theileriosis and babesiosis infection while the old age groups due to the reinfection to one time or more developed a good immune defense that gives the animal resistance against *Theileria annulata*. [12,20,21,26]. On the other hand high percentage bovine anaplasmosis infection in old age groups and lower percentage in small age groups this agreement with [20,21].

There was significant difference between the prevalence of theileriosis, babesiosis and anaplasmosis in crossbred cattle which higher than native cattle this attributed to the difference in management practice where the crossbred or exotic breeds receives more attention in terms of ectoparasite control and feed supplementation. Therefore the breed susceptibility of theileriosis, babesiosis and anaplasmosis recorded in this study support the report of [12,20,21, 27].

The prevalence of hemoparasitism was higher in female than male animals possible due to the fact that females are kept much longer for breeding and milk production purposes this agreement with [27]. Decrease in hemoglobin concentration Hb, Packed cell volume PCV, and erythrocyte count RBCs attributed to most hemoparasites reported in this paper are known to replicate in the erythrocytes leading to hemolysis and anemia. Whenever, total leukocyte count and lymphocyte was showed a mild increase in theileriosis and slight increase of eosinophils this agreement with [12,13,28].

CONCLUSION

The area of study is endemic with theileriosis, babesiosis and anaplasmosis infection. The prevalence of hemoparasite infection is influence by season period. of feeds The presence of carrier populations of hemoparasite infected cattle which both serve as a reservoir of infection for tick-vectors and susceptible livestock, and has the potential for clinical relapse under stressful conditions.

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