Prevalence of piroplasmosis (Theileriosis and Babesiosis) among goats in Duhok Governorate

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Abstract

The prevalence of *Theileria hirci* and *Babesia motasi* was studied in goats in the Duhok area of Iraq from April to September 2010. A total of 500 local black breed goats represent 35 flocks of different localities were clinically and then in laboratory examined for the presence of piroplasmosis in blood smears. The study revealed that 20.8% of the goats infected with *T. hirci* and 4% infected with *Babesia motasi*. Prevalence of piroplasms infection between male and female and between different age groups of goats was statistically significant. Hematological findings, showed a significant decrease in red blood cell, hemoglobin concentration, and packed cell volume beside the significant increase in the mean corpuscular volume. No changes in mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration values were noted. Macrocytic and normochromic type of anemia was found.

انتشار داء الكمثريات (ثايلريا وبابيزيا) في الماعز المحلية في محافظة دهوك/ العراق

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الخلاصة

أجريت هذه الدراسة لمعرفة انتشار داء الكمثريات Caprine piroplasmosis في المعز المحلية، خلال الفترة من شهر نيسان إلى شهر أيلول لسنة 2010، حيث تم فحص 500 من المعز المحلي مثلت 35 قطيعا منتشرة في مناطق مختلفة في محافظة دهوك/ العراق. استخدمت المسحات الدموية الخفيفة والمصبوغة بصبغة الكمزا لغرض تشخيص العامل المسبب. أوضحت نتائج الدراسة إن نسبة إصابة المعز بمرض Piroplasmosis كانت 24.8%. حيث تم تشخيص نوعين من الطفيلي هما *Babesia motasi* and *Theileria hirci* في المعز المصابة وكانت نسبة الإصابة 20.8% و4% على التوالي. كما سجلت فروقات معنوية في معدل إصابة المرض بين الذكور والإناث، وبين أعمار مختلفة تحت مستوى ($p<0.05$). أظهرت نتائج الدراسة أن الخمج بطفيليات الدم *Babesia motasi* and *Theileria hirci* كان له تأثير واضح على المعايير الدموية للمعز المخمج، حيث لوحظ تناقص معنوي في معدلات العدد الكلي لكريات الدم الحمر، وحجم خلايا الدم المرصوصة، وتركيز خضاب الدم، تحت مستوى ($p<0.05$). كما بينت نتائج هذه الدراسة زيادة في معدل الحجم الكروي MCV تحت مستوى ($p<0.05$)، في حين لم تظهر أي فروقات معنوية في معدلات خضاب الدم الكروي MCH ومعدلات تركيز خضاب الدم الكروي MCHC وقد تبين أن فقرا لدم كان من النوع ذي الكريات كبيرة الحجم سوية الصباغ Macrocytic normochromic anemia.

Introduction

Ticks and tick borne diseases (TTBDs) are widely distributed throughout the world particularly in tropical and subtropical countries. Endemic diseases such as tick-borne diseases particularly theileriosis, babesiosis and anaplasmosis which have considerable economic importance locally and regionally but are non–threatening internationally (1,2). In Iraqi, Caprine piroplasmosis is caused by *Theileria hirci (lestoquardi)* and *Babesia motasi*. However, little is known about the epidemiology and socio-economic impact of piroplasmosis in this region. Piroplasmosis, caused by *Theileria* and *Babesia spp*, is a common complex disease in tropical and subtropical regions since it affects wide range of ruminant and cause severe economical losses (3). Malignant theileriosis of sheep and goats is usually acute and highly fatal disease of adult animals manifested by high fever associated with nasal discharge, jaundice, enlargement of superficial lymph nodes and sometimes hemoglobinurea (4). The disease may be highly fatal and mortality rate ranging between 50-100% in endemic areas while the nature of the disease in lambs and kids is mild and this possibly due to maternal immunity (5). Ovine babesiosis is the most important hemoparasitic tick-borne disease of small ruminants caused by *Babesia ovis*, *Babesia motasi* and *Babesia crassa*. These parasites are widespread in tropical and subtropical areas of the world (6). *B. ovis* is highly pathogenic especially in sheep and causes severe infections which are characterized by fever, anemia, icterus and hemoglobinuria. Mortality rates in susceptible hosts range from 30 to 50% in infected fields. The pathogenecity of *B. motasi* is not high and appears to be moderately virulent. In contrast, *B. crassa* is considered as being non-pathogenic to small ruminants (7, 8). Microscopic examination of Giemsa stained blood smears remains the most appropriate for the diagnosis of acute babesiosis, but the low sensitivity of the method does not permit its use in epidemiological investigations (9). The aims of present study includes: Estimate the prevalence of piroplasmosis (Babesiosis and Theileriosis) among goats in Duhok province and evaluation of hematological change of acute piroplasmosis in infected goats.

Materials and Methods

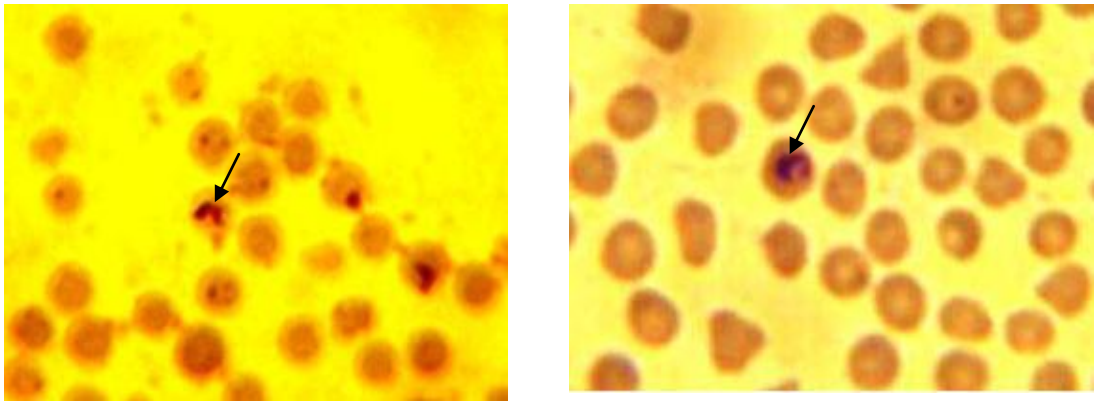
- **Sampling:** The prevalence of piroplasmosis **(**Theileriosis and Babesiosis) was studied in goats in Duhok area, North Iraq from April to September in 2010.A total of 500 local breed black goats, males 74 and female 426 and the age was varied from Less than one year to Above 3 years from 35 flocks of different localities (districts and sub districts) which include Zakho, Batel, Sumaill, Aqra, Bamerny, Sarseng and Tenahi. The animals were examined clinically and subjected to laboratory examination to detect for the presence of *Theileria* and *Babesia species* and by appropriate Giemsa stained blood smear.
- **Blood Smears:** Method of preparation of blood smears and Giemsa staining was used as described by (10) for detection of the Theileria and Babesia specie. The thin and thick blood smears were prepared from the peripheral ear vein. Before taken of the sample, the site was clipped and wiped with 70% ethanol and then punctured and allowed to dry, the first drop of blood always discarded. A drop of blood was taken on a clean glass microscope slide, spread by another slide at an acute angle, air dried and fixed by absolute methanol for 5 minute and stained with 10% Giemsa stain. The film was examined by light microscope using 1000x oil immersion.
- **Hematological examination:** Five milliliter of blood was drained from jugular vein puncture, after preparation of the area aseptically, by clipping and then soaking with 70% alcohol, mixed in disposable clean plastic tube with an anticoagulant (EDTA) and used for the estimation of some hematological parameters including total

erythrocyte count (RBC) by hemocytometer method, packed cell volume (PCV) by hematocrit method (Microhematocrit method), hemoglobin concentration (Hb) by acid hematin method (Sahli method), in addition to hematological indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) as described by (11).

- **Statistical analysis:** The results were analyzed by chi- square test (X^2) (12).

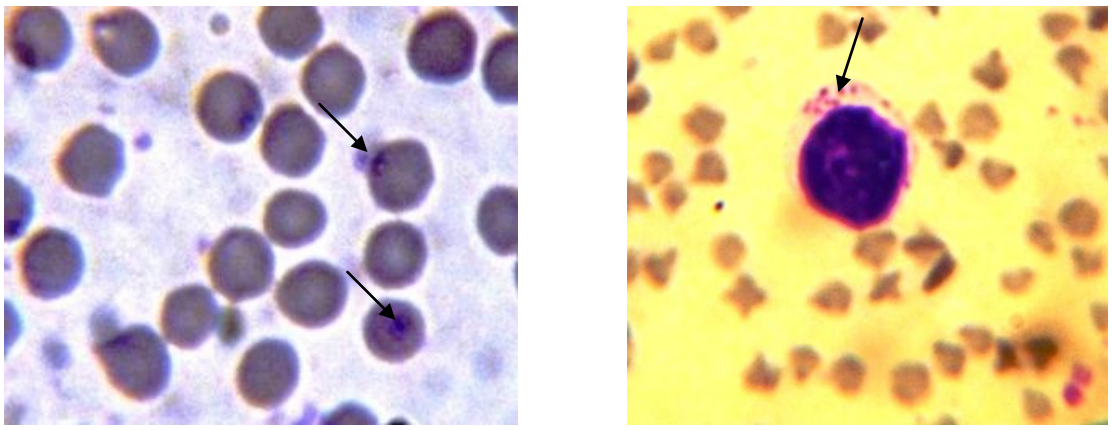
Results

- **Morphology:** Depending on morphological characteristics of the merozoite in infected erythrocytes by Giemsa stained blood smear, Both *Babesia motasi* and *Theileria hirci* were identified by light microscopic examination. *B. motasi* appeared as a large body, single or double pyriform, formed an acute angle inside erythrocytes. Piroplasms of *T. hirci* appeared as a small oval, round or dotes- like inside erythrocytes as and machroschizont in large lymphocyte as shown in Fig (1,2)



A. Ring form **B. Pyriform form**

Fig. (1) Morphological forms of *Babesia motasi* within infected erythrocytes



A **B**

Fig. (2) *Theileria hirci* within RBC (A) and Machroschizonts form of *Theileria hirci* within a lymphocyte in Giemsa stained blood smears (B) (x1000)

- **Prevalence:** The prevalence of piroplasmosis in goats determined by Giemsa stained blood smears as shown in Table 1. of the 500 native goats blood smears 124 (24.8%) was positive for piroplasmosis in all districts and sub districts. The highest rates of infection were in Aqra 30 (56.6%) and a lowest rate was in Batel 19 (15.6 %). In other areas, the rate was in Zakho 36 (25.7%), in Tenahi 17 (24.3%) and in Sumail 22 (19.1%).

Table (1) The prevalence of piroplasmosis in goats in different districts and sub districts of Duhok province

Area	Number of sample examined	Number of +ve	%
Zakho	140	36	25.7
Aqra	53	30	56.6
Batel	122	19	15.6
Sumail	115	22	19.1
Tenahi	70	17	24.3
Total	500	124	24.8

The Prevalence of *Theileria hirci* and *Babesia motasi* in goats in different districts and sub districts as shown in Table 2. The morbidity rate was 104/500 (20.8%) for *Theileria hirci* and 20/500 (4%) for *Babesia motasi*.

Table (2) Prevalence of *Theileria hirci* and *Babesia motasi* in goats in different districts and sub districts of Duhok province

Area	No. of sample examined	No. of animals +ve to piroplasmosis	No. of +ve to *Theileria hirci*	%	No. of +ve to *Babesia motasi*	%
Zakho	**140**	36	26	20.96	10	8.06
Aqra	**53**	30	25	20.16	5	4.03
Batel	**122**	19	18	15.51	1	0.81
Sumail	**115**	22	20	16.12	2	1.61
Tenahi	**70**	17	15	12.09	2	1.61
Total	**500**	124	104	84.84	20	16.12

The prevalence of Theleria varied from 54.8% in age above 3 years to 5.6% in the age less than one year, while was 23.4% in the age 1-3 years. In prevalence of Babesia was also varied from 8.9% in age above 3 years to 1.6% in the age less than one year, while was 5.6% in the age 1-3 years as shown in Table 3.

Table (3) Prevalence of *Theileria hirci* and *Babesia motasi* in relation to the age of goats

Age	No. of animals examined	No. of +ve to *Theileria hirci*	%	No. of +ve to *Babesia motasi*	%
Less than one year	70	7	1.4	2	0.4
1 – 3 years	165	29	5.8	7	1.4
Above 3 years	265	68	13.6	11	2.2
Total	500	104	20.8	20	4

The differences between prevalence rates of *Theileria hirci* and *Babesia motasi* were statistically significant between male and female. In *Theileria hirci* the rate was 97 (19.4%) in female and 7 (1.4%) in males while in *Babesia motasi* the rate was 18 (3.6%) in female and 2 (0.4%) in males as shown in Table (4).

Table (4) The prevalence of *T.hirci and B. motasi* in goats according to the sex

Sex	No. of samples examined	No. of +ve to *T.hirci*	(%)	No. of +ve to *B. motasi*	%
Male	74	7	1.4	2	0.4
Female	426	97	19.4	18	3.6
Total	500	104	20.8	20	4

*** Statistically significant at a level of (P< 0.05)**

- **Hematology:** The hematological findings of the goats naturally infected with Piroplasmosis are shown in Table 5. The results of the study indicate that the values of blood parameters varied in comparison with normal values of blood pictures in

the healthy animals. The PCV mean value was (19.7± 2.66% and 6 ± 0.88% in Theilerial and Babesial infection respectively. The hemoglobin concentration g/dl mean value was 6 ± 0.88 in Theilerial infection and 6.4 ± 0.93 in Babesia infection. The infected goats showed 5.8 ± 0.93 million cell/µL mean value of the total number of erythrocytes in Theilerial infection and 6 ± 0.88 million cell/ µL in Babesial infection. The mean value of the MCV was 34.1 ± 4.36 fl and 32.2 ± 4.86 fl in Theilerial and Babesial infection respectively, and MCH in goats infected with Theileiosis was 11 ± 1.44 pg while in Babesial infection 10.7 ± 1.72 pg and MCHC was 32.4 ± 2.21 g/dl and 32.6 ± 1.71 g/dl in Theilerial and Babesial infection respectively, this indicates the evidence of macrocytic normochromic anemia.

Table (5) Hematological parameters of goats infected with piroplasms (*T. hirci* and *B. motasi)*

Parameters	*T. hirci* **(Mean ± S.D)**	*B. motasi* **(Mean ± S.D)**
Total RBC X 10^6 / µL	5.8 ± 0.93 [a]	6 ± 0.88 [a]
PCV %	19.7± 2.66 [b]	19.6± 3.11 [b]
Hb g/dl	6 ± 0.88 [c]	6.4 ± 0.93 [c]
MCV (fl)	34.1± 4.36 [d]	32.2± 4.83 [d]
MCH (pg)	11± 1.44	10.7± 1.72
MCHC g/dl	32.4 ± 2.21	32.6 ± 1.71

Discussion

Little known about piroplasmosis in Iraq particularly in small ruminants (sheep and goats) and scarce data regarding the prevalence and occurrence of such diseases in northern region of Iraq. Accordingly, the objectives of this study were to determine the prevalence of piroplasmosis (*Theileria and Babesia Spp*) in Duhok province. In this survey, the Giemsa stained blood smears was applied to study the prevalence of infection from different flocks of goat's district and sub district. For the diagnosis of sheep piroplasmosis in Iraq early studies were mainly based on microscopic examination (13). The most common diagnostic method for identification of piroplasmosis involving Giemsa staining of blood smears (14). The diagnosis of tick borne diseases such as babesiosis, theleriosis and anaplasmosis still depends on observing the parasities in the infected erythrocytes when the parasitemia rates are indicative. Although the parasite detection can be easily applied in the field, the sensitivity of the method and its failure to detect piroplasmosis, if the number of parasite in the peripheral blood is too low, illustrate the limitation of parasitological diagnosis by Giemsa stained blood smears (5). In this study the results showed prevalence rate 124 (24.8%) of piroplasmosis among goats in all areas of examination with variable rates from district to other ranged from 25.5% to 56.6%. Both *T. hirci* and *B. motasi* detected by Giemsa stain in infected goats with various incidence rates 20.8% infection rate with *T. hirci* and 4% with *B. motasi* from 500 samples. (15) Found that the incidence of protozoal disease among sheep and goats was 7.5% in Mosul province, and (16) revealed the rate of piroplasmosis was 8.6% in Syria. (17) Reported that the prevalence of *T. hirci* in goats in Baghdad was 33.82%. (18) Revealed that the prevalence rates of *T.hirci* and *B. motasi* in goats in Mousl province in Iraq were 28.86% and 30.92% were infected with *T.hirci* and *B. motasi* respectively. The variability in the prevalence among districts was due to close relationship between incidence of piroplasmosis and activity period and distribution area of the ticks. The *Theileria hirci* was appeared in infected erythrocyte as oval, rounded, small rod and large dote like, these form and shapes of parasites were similar to those described by

(5,19). (20) Reported *T. hirci* in sheep from Arbil province for first time. (21) Found that the piroplasms form of *T. hirci* could be seen in the erythrocyte of artificially infected sheep and were oval and round forms, but naturally infected sheep showed parasitemia ranging from 3.2% to 3.7%. (22) Found *T. hirci* was found in one lymph smear out of 36 sheep (2.8%) in Jeddah and 6 out of 25 sheep (24%) in Buriede. An interesting finding by (13) is that none of the sheep in Arbil province were infected and there was a relatively low incidence of infection in other two Northern provinces, Suliamanyia and Mosul, where the rates were 7.33% and 15% respectively. Also (23) isolated Theleria and Babesia from naturally infected sheep from Mosul province. (24) Revealed a mean sero-prevalence of 59.9% of *T. hirci* in Awassi breed sheep in Syria. (25) Found (36.17%) of sheep in Iran were infected to *T. hirci* with parasitemia of 0.01-15%. Also the protozoan *Babesia motasi* was noted in microscopic examination revealed presence of *B. motasi* in many erythrocytes and the appearance of parasite was variable from single to double pyriform these shapes are similar to those described by (5, 19). (13) Isolated *B. motasi* from natural infected sheep in Baghdad province. (25) Found 23.5% and 14% of sheep and goats in Iran were infected with *B. ovis* and *B. motasi*, respectively. (26) Found the maximum range of infection of *B. motasi* from 15% to 90% in India. (27) Observed *B. motasi* by thin blood films to much more common than *B. ovis* prevalence rates were in average about 20% in Nigeria goats. The prevalence rate of piroplasmosis and incidence of both protozoan *B. motasi* and *T. hirci* related to age groups of goats was studied. A high rate was in age group above 3 years in comparison to other age groups. Under one year and 1-3 years, this event was in agreed with (28) whose found that age, sex, and breed influenced the prevalence of protozoa diseases. (39) Found that the age of host influences its susceptibility to infection and therefore affects the incidence of disease in a population of animals infected with Babesia with age from about 6 month. However this might be role of passive immunity protection which can be induced via colostrums in first age groups and recovery from acute phase of infection result premunity prevent challenge infection. (21) obtained the incidence rate of *T. hirci* infection in lambs and adult sheep were high and similar 17.12% but it was low in adult goats 8.06% in comparison with young goats which was a high rate 40% and (25) found the prevalence of theleriosis in all age group and was statistically non significant. (17) Found infection rate of *T. hirci* was high in goats aged 4 years and also infection seen in kids 45 days old. Thus new born lambs might contract *T. hirci* infection due to strains that differ from those which challenge their dams (30). The differences in prevalence rate have been found by other studies, it may be due to different in climate which had effect on distribution the vector specially hard ticks from area to other area and also might be the role of natural immunity in exposure to infection. In this study revealed variable degrees in hemograms in goats infected with *T. hirci* and *B. motasi* confirmed that severe hemolytic anemia was very distinctive when in comparison to normal healthy parameters. These included a drop in the mean values of erythrocyte count, packed cell volume and hemoglobin concentration and also there was a significant increase in the mean values of the mean corpuscular values (MCV), while there was no difference in (MCHC, MCH). This indicated evidence of macrocytic normochromic anemia in both infections. This involves the development of intravascular hemolysis which is directly proportional to parasitemia. (18) Indicated that blood parasitic infection of blood parameters as total red blood cells, haemoglobin concentration, packed cell volume significantly decreased beside the significant increase in the erythrocyte sedimentation rate (ESR), and also found macrocytic hypochromic anemia in Theileria and Babesia infection. (31) described the hematological changes in theilerial infection which occur by direct and

indirect effects of schizogony on the lymphocyte and direct effects possibly toxic on the bone marrow, and also thought to be due to removal of infected erythrocytes by spleen and liver and due to destruction of red cell by intra-erythrocytic stages and greater red cell removed by *Theileria spp* result in bilirubinemia and bilirubinuria decreased in erythrocyte count, packed cell volume and hemoglobin in *T. hirci* infection were observed workers (23). (30) Classified the anemia in *T. hirci* infection as hemolytic anemia due to direct destruction and role of auto-haemagglutination antibodies. (30) Found normocytic normochromic anemia with *T. hirci.*

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