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Seroprevalence of piroplasmosis with tick distribution in northern Iraq

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Abstract

The current study was carried out on blood samples of 299 local breed female cattle in Erbil, Duhok and Suleimania, Northern Iraq, for the period from beginning of January till end of December 2006 for detection of piroplasmosis. By direct blood smear examination, the rate of *Theileria annulata* alone was 45.1% while in mixed infections with Babesia was 11.7%. The total rate of Theileria infection was 56.9%. The haematological parameters of cattle infected with Theileria alone were PCV=27%, RBC= 5.6 million/ cm^3 and Hb 9.5 g/liter did not vary from non infected ones. While in mixed infections (Theileria +Babesia) the blood picture values were decreased dramatically and were PCV=18%, RBC=4.08 million / cm^3 and Hb 5.7 g/l. Using enzyme linked immunosorbent assay technique (ELISA), the seropositivity of Theileria was 77.9%, while Babesia was 12.4%. The overall rate of seropositivity by ELISA was 90.3% for piroplasms while by blood smears examination the rate of infected animals was 56.9%. From 5804 ticks collected from animal body, the constituencies of the ticks were 81.7% *H. anatolicum anatolicum*, 15.3% *H. marginatum marginatum*, 2.82% *Rhipecephalus appendiculatus* and 10 ticks (0.2%) were not identified. The highest rate of ticks was found attached to udder and under tail (77%), followed by ears (20%) and hind limbs and around eyes (3%). The distribution of ticks was highest in spring 96.0%, followed by summer 4.0%. No ticks were detected in winter and autumn. The greatest number of ticks was in March (37.9%) followed by May (32.23%), April (25.85%), June (2.17%), July (1.68%) and August (0.17%).

Keywords: Piroplasmosis; ticks; Iraq
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نسبة الانتشار المصلي لداء الكمثريات في شمال العراق

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الخلاصة

تضمنت الدراسة فحص ٢٩٩ بقرة محليةحيث شملت ثلاثة محافظات من العراق (اربيل، دهوك، السليمانية). للفترة من بداية كانون الثاني علم و استمرت حتى نهايته كانون الاول علم ٢٠٠٦. و ذلك باستخدام طريقة المسحات الدموية الرقيقة و طريقة الفحص المصلي و هي طريقة فحص ELISA. تبين من خلال الدراسة ان نسبة الاصابة بطفيلي *Theileria annulata* قد وصلت الى ٤٥,١% بينما لم يلاحظ وجود طفيلي *Babesia bigemina* كاصابة منفردة و لكن سجل مع الطفيلي السابق كاصابة منفردة و قد كانت نسبة الاصابة ١١,٧% بأستخدام طريقة المسحة الدموية الرقيقة. النسبة الكلية للـ *Theileria annulata* كانت ٥٦,٩ %. اما دراسة التغيرات الدموية في الابقار المفحوصة بينت عدم وجود أي فرق معنوي مقارنة مع المعدلات الطبيعية للعدد الكلي لكريات الدم الحمر٥,٦ مليون /ملم^3، كمية خضاب الدم ٩,٥ غم/ ملم^3 و حجم كريات الدم المرصوصة ٢٧% عند الاصابة بطفيلي *Theileria annulata* بينما لوحظ انخفاض ملحوظ في هذه المعدلات في الحيوانات المصابة بكلا الطفيليين *Theileria annulata* and *Babesia bigemina* . (كريات الدم الحمراء ٤,٠٨ مليون/ ملم^3، حجم كريات الدم المرصوصة ١٨%، وخضاب الدم ٥,٧ غم/ ملم^3) اما نتائج الفحص بطريقة ELISA فكانت نسبة الاصابة بطفيلي *Theileria annulata* هي ٧٧,٩% بينما كانت نسبة الاصابة المختلطة بطفيليات *Theileria annulata* and *Babesia bigemina* وهي١٢,٤% وان نسبة الاصابة الكلية كانت ٩٠,٣% بينما بواسطة فحص الدم المباشر كان ٥٦,٩%. تم جمع ٥٨٠٤ نموذج من القراد الصلب و لكلا الجنسين من الذكور و الاناث. كانت نسبة الاصابة بالنوع *Hyalomma anatolicum anatolicum* ٨١,٧% و بالنوع *Hyalomma marginatum marginatum* كانت ١٥,٣% و اما *Rhipecephalus appendiculatus* كانت قد وصلت ٢,٨٢% و١٠ قراد لم

يشخص (٠,٠٢%). اما تواجد القراد على الحيوان فكانت بنسبة ٧٧%، ٢٠%، ٣% في منطقة الضرع و تحت الذيل و الاذن على التوالي. نسبة انتشار القراد كانت اعلى في فصل الربيع حيث بلغت ٧,٩% قد جمعت في شهر اذار و في شهر مايس ٣٢,٢٣% و في كل من الاشهر نيسان و حزيران و تموز و اب قد وصلت نسبة تواجد القراد الى ٢٥,٨٥%، ٢,١٧%، ١,٦٨%، ٠,١٧% على التوالي.

Introduction

Piroplasmosis, caused by Theileria or Babesia, is one of the most common complexe diseases in tropical and subtropical regions since it affects wide range of ruminants and causes sever economical losses (1). Bovine piroplasmosis is caused by *Theileria annulata* and *Babesia bigemina* is common problem in the north of Iraq. However, little is known about the epidemiology of piroplasmosis in this region.

Hassan (2) collected blood samples from 250 cattle (50 tick-infested, 50 tick non-infested and 150 farm cattle) during the period from June 2007 to August 2008 to detect Mediterranean theileriosis in Sulaimania region. He found the highest frequency in tick infested cattle 54%, followed by farm cattle 21.3% and the lowest in tick non-infested cattle 6%, by Giemsa stained blood smear examination.

Generally, the diagnosis of clinical infection by piroplasmosis in cattle is usually based on clinical signs, history of disease and vector distribution and identification of the piroplasm and schizont stages in blood and lymph node smears. Carrier animals, in which low numbers of erythrocytes remain infected, are important contributors to the transmission of the infection by tick bites. Hence, detection of piroplasms in carrier animals is very important to control the infection. However, detection of piroplasms is not easy and it is generally not possible to discriminate pathogenic and non-pathogenic species that may occur simultaneously within the same host (3,4).

There are several techniques for serodiagnosis of piroplasmosis such as agglutination test, Immunofluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA). Principally they are easy to perform and proved to be powerful diagnostic and research tools (5).

ELISA has been successfully adapted for the detection of antibodies to piroplasmosis and has been shown to detect antibodies for a longer period time than the indirect fluorescent antibody test (6). It is easy to perform, inexpensive, accessible to be standardized and would fulfill the requirement for epidemiological surveys when compared to the IFAT (7).

The present study was planned to show the prevalence of Babesia and Theileria spp. And the distribution of tick vector in Duhok, Erbil and Suleimania governorates, Northern Iraq.

Materials and methods

The study was conducted during the period from beginning of January to end of December 2006 at different localities in Duhok, Erbil and Suleimania provinces of the Northern Iraq. The investigated area extended from the latitude 33N to the latitude 38N and longitude 42 E to longitude 46 E. This area is distinguished by Mediterranean mountains climate in the winter and desert dry climate in summer.

The survey study was carried out on randomly selected local breed, outdoor grazing apparently healthy female cattle's. The study involved 299 cattle, 100 from each Dohuk and Suleimania governorate and 99 cattle from Erbil governorate. Age of the cattle was above one year old.

Blood samples were collected from jugular vein, divided into two portions. One portion was put in plain vacationer tube, centrifuged and sera were stored at -20 C, while the second portion of blood put in the heparinized vacationer was used for estimation of PCV, RBC and Hb in addition to that blood smears were prepared from each sample and stained with Giemsa stain (8).

Enzyme-linked immunosorbent assay (ELISA) for *Theileria annulata* was carried on using *Theileria annulata* surface protein "TaSP" following the protocol described by Bakheit, *et al.*, (9). Enzyme-linked immunosorbent assay for *B. bigemina* was conducted using ELISA kit (Svanova, Sweden) according to the instruction in the manual.

Total body collection of ticks was made from cattle using a pair of blunt forceps. Ticks were identified according to Walker *et al.* (10). The identification was confirmed by Iraqi National Museum –Baghdad.

Statistical analysis was conducted using SPSS. Chi-Square analysis was used to show significant difference between groups at level 5% (11).

Results

In Giemsa-stained blood smears, from total of 299 cattle, 135 (45.1%) were positive for Theileria, 35 (11.7%) were positive for mixed infections of Theileria and Babesia. No positive cases were found for Babesia alone.

The distribution of Theileria *was* similar in Duhok and Suleimania, where 44 animals (44%), while 47/99 (47.4%) was found in Erbil. Regarding the mixed infection the following results were reported: 12.1% in Erbil, 13% in Duhok and 10% in Suleimania.

The haematological parameters did not vary significantly between normal and Theileria infected animals,

while declined substantially in mixed infections of Theileria and Babesia (Table 1).

Theileria annulata specific antibodies were detected in sera of 233 animals out of 299 (77.9%), when these samples were tested using ELISA based on a recombinant protein from *Babesia bigemina*, a total of 37 animals (12.4%) were positive, which were also positive for *Th. annulata* (Table 2).

A comparison between the efficiency of ELISA and microscopical examination clearly showed that ELISA was significantly more efficient for detection of positive cases than direct microscopic examination. Hence, ELISA allowed a better detection (90.3%) of infected animals, while microscopically examination detected only 56.9% of the cases (Table 3).

A total of 5804 ticks (4350 males and 1454 females) was collected and characterized according to the key for identification of Ixodidae. It was found that 4740 (81.7%) were *Hyalomma anatolicum anatolicum* and 890 (15.3%) *Hyalomma marginatum marginatum* while *Rhipicephalus appendiculatus* constituted only 164(2.8%) and 10 ticks were not identified (Table 4).

Table 1: The PCV, RBC count and Hb values of infected and non-infected cattle.

Parameters	Normal*	*Theileria annulata*	Mixed infection Theileria+Babesia	Non-infected
PCV %	6-42%	27±0.022	18±0.034	30 ±0.046
RBC 10^6/ml	5-8 $\times 10^6$	5.6±0.25	4.09±0.64	5.6±0.53
Hb g/l	8-14	9.5±0.66	5.7±0.635	10.6±1.13

* Coles (12) P<0.05.

Table 2: Sero-prevalence of *T. annulata* and *B. bigemina* infection from 299 samples.

Infection	*Theileria annulata* alone	*Babesia Bigemina*	Babesia and Theileria Mixed infection	Total
No. Positive	233	0	37	270
Positive %	77.9	0	124	90.3

Table 3: Comparison between ELISA and blood smears for the diagnosis of Theileria and Babesia infections.

Infections	Blood smear		ELISA test	
	Number	%	Number	%
Theileria annulata	135	45.1	233	77.9
Mixed infected	35	11.7	37	12.4
Total positive	170	56.9	270	90.3
Total negative	129	43.1	29	9.6

Table (4) Distribution of ticks according to species.

Tick species	*Hyalomma spp.*		R. appendiculatus	Unknown
	anatolicum	marginatum		
Number	4740	890	164	10
Percentage (%)	81.7%	15.3%	2.8%	0.2%

It was found that 4468 (77%) ticks were attached to the udder and to under tail, whereas 1161 (20%) were attached to the ears and 175 (3%) on the hind limbs and around the eyes. No ticks were seen on the chest of the examined cows (Table 5).

The distribution of ticks in the region was highest in spring (96.0%) followed by summer (4.0%). No ticks were detected in winter and autumn. The higher number of ticks could be detected in March 2200 (37.9%), followed by May 1870 (32.23%), April 1500 (25.85%), June 126 (2.17%), July 98 (1.68%) and August 10 (0.17%).

Table (5). Tick distribution on different parts of the animal body.

Locations	Tick number	Percentage
Udder and under tail	4468	77
Ears	1161	20
Hind limb	125	2
Around eyes	50	1
Total	5804	100

Discussion

In the present study 56.9% of cattle were found infected with *Theileria annulata* and 11.7% of them were associated with Babesia infection by microscopic examination of Giemsa stained blood smears. While 91% of cattle had antibodies against Theileria and mixed infections of Theileria and Babesia using TaSP recombinant ELISA (9). The lower rate of infections in direct blood smears examination reflects the low sensitivity of microscopic examination of blood smears for detection of Theileria and both Theileria and Babesia infections (13).

The PCV, RBC count and Hb values of negative animals and *Theileria* infected animals were almost identical. These values were substantially reduced in animals infected with both Theileria and Babesia. This may be due to *Babesia* amplified the pathogenesis of the mixed infection.

The results revealed that 91% of the tested cattle produced antibodies against the TaSP recombinant protein indicating that these animals experienced an infection with *Th. annulata*. Moreover, these data indicated that the region from where the samples were collected can be considered as an endemic region regarding tropical theileriosis.

Regarding the suitability of TaSP-ELISA used in this study, investigation was performed to validate this test under field conditions. Salih *et al*. (14) tested more than 800 serum samples and found that TaSP-based ELISA had higher sensitivity than a number of other ELISA tests based on the use of Tams-1 recombinant protein or crude *Theileria* material. Taken together these data, TaSP was a useful serological tool for epidemiological studies.

Assessment of antibodies against *B. bigemina,* was conducted using ELISA kit (Svanova®, Sweden). In 12.4% of the tested animals, mixed infections with Theileria and Babesia were found. These study of Camus and Montenegro-James (15), observed in a previous study that the incidence of babesiosis ranged between 18% and 71%, as detected by ELISA. The difference in the rate of infection may be related to location and environmental variation in two studies.

Finding of both *Hyalomma antolicum antolicum* and *Hyalomma marginatum marginatum* were also reported by earlier studies in Iraq (16). The highest rate of ticks were attached to udder and under tail followed by ears. In Sulaimaniyah province (17) found *Hyalomma anatolicum anatolicum, H. marginatum, Rhipicephalus turanicus* and *R. sanguineus* in sheep.

It is concluded that theileriosis is more widely distributed than babesiosis in northern Iraq. ELISA method was more sensitive than microscopical examination of Giemsa-stained smears. The distribution of Hyalomma ticks was greater than Rhipicephalus. Both tick species were predominant in spring and early summer.

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