

## Seroprevalence of *Babesia bigemina* and *Anaplasma marginale* in domestic animals in Erbil, Iraq

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### Abstract

Seroprevalence of *Babesia bigemina* and *Anaplasma marginale* in cattle, sheep, goats and wild goats was studied in Erbil district, between January to December 2010. A total of 184 blood samples were collected from 44 cattle, 59 sheep, 70 goats and 11 wild goats for the preparation of blood smears and serum samples which tested against *B. bigemina* and *A. marginale* using the SVANOVIR<sup>®</sup> *B. bigemina*-Abs and *A. marginale*-Abs ELISA Kit. The overall prevalence of *B. bigemina* infection was 12 (27.27%), 4 (6.77%), 5 (7.14%) and 1 (9.09%) in cattle, sheep, goats and wild goats and for *A. marginale* 4 (9.09%), 2 (3.38%), 3 (4.28%) and 1 (9.09%) respectively. The co-infections between *B. bigemina* and *A. marginale* were 25% in cattle, 33.33% in sheep, 37.50% in goats and 50% in wild goats. The seasonal prevalence of *B. bigemina*, *A. marginale* and co-infection between them peaked in both spring and summer as revealed by blood smear examination and ELISA.

**Keywords:** *Babesia bigemina*, *Anaplasma marginale*, Domestic animals, ELISA.

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### التحري مصليا عن الباييزيا و الأنابلزما في الحيوانات الأليفة في أربيل، العراق

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### الخلاصة

تم التحري مصليا عن *Babesia bigemina* و *Anaplasma marginale* في الماشية والأغنام والماعز الأليف والبري في محافظة أربيل للفترة من كانون الثاني ٢٠١٠ ولغاية كانون الأول ٢٠١٠، اذ جمع (١٨٤) عينة دم من (٤٤) مواشي و(٥٩) اغنام و(٧٠) ماعز أليف و(١١) ماعز بري وذلك لتحضير مسحات الدم وعينات المصل التي اختبرت للتحري عن الأجسام المضادة للـ *B. bigemina* و *A. marginale* بطريقة اليزا وحسب عدة العمل المجهزة من شركة. بلغ انتشار الإصابة بـ *B. bigemina* بنسبة 12 (27.27%)، 4 (6.77%)، 5 (7.14%) و 1 (9.09%) في الماشية والأغنام والماعز والماعز البري على التوالي. أما نسبة الإصابة بـ *A. marginale* فقد بلغت 4 (9.09%)، 2 (3.38%)، 3 (4.28%) و 1 (9.09%). كانت نسبة الإصابة المشتركة بين *B. bigemina* و *A. marginale* (25%) في الماشية و(33.33%) في الأغنام و(37.5%) في الماعز وبنسبة (50%) في الماعز البري، في حين وصلت ذروة السيادة الفصلية للإصابة بكليهما وبإصابتهما المشتركة في فصلي الربيع والخريف كما أوضحتها فحوصات مسحة الدم واختبار الاليزا.

### Introduction

Tick-Borne Diseases (TBDs) are a constraint to livestock production in many developing countries of the world. They are responsible for high morbidity and mortality resulting in decreased production of meat, milk and other livestock products and the loss of draught power,

They are also a significant impediment to the improvement of indigenous breeds of cattle, sheep and goats, since they prevent the introduction of more productive exotic breeds (1). Ticks transmit a greater variety of pathogenic microorganisms, than any other arthropod vector group, and are among the most important vectors of diseases affecting livestock. In general, tick-borne protozoan diseases (e.g.

Theileriasis and Babesiosis) and rickettsial diseases (e.g. Anaplasmosis and Heartwater or Cowdriosis) are pre-eminent health and management problems of cattle, small ruminants and buffaloes, affecting the livelihood of farming communities in Africa, Asia and Latin America (2).

Babesiosis is a worldwide tick-borne hemoprotozoosis affecting many mammalian species and caused by intraerythrocytic multiplication of apicomplexans in the *Babesia* genus. The evolutionary success of this parasite is attested by the large number of species described more than 100, with numerous species probably remaining to be discovered and/or described (3). Babesiae are the second most common blood-borne parasites of mammals after the trypanosomes. More than 100 species of Babesiae have been identified which are traditionally divided on the basis of their morphology into the small and large groups. To date, only ixodid ticks have been identified as vectors for *Babesia* spp. The specific tick vector must feed on a vertebrate reservoir that is competent in maintaining the *Babesia* organisms in an infectious state. (4).

Anaplasmosis is an arthropod-borne, haemolytic disease of ruminants caused by the rickettsial haemoparasite, *A. marginale* (5). *A. marginale* is the most prevalent tick-borne pathogen of animals worldwide and is responsible for severe morbidity and mortality in temperate, subtropical, and tropical regions (6). Anaplasmosis reduces the animal's body weight, reduces milk production, causes abortions, and frequently leads to death (7 and 8). *Anaplasma* spp transmitted by at least 20 ticks' species, including *Argas persicus*, *Ornithodoros lahorensis*, *Boophilus annulatus*, *B. decoloratus*, *B. microplus*, *Dermacentor albipictus*, *D. andersoni*, *D. occidentalis*, *D. variabilis*, *Hyalomma excavatum*, *Ixodes ricinus*, *Rhipicephalus bursa*, *R. sanguineus* and *R. simus* (9). The aim of the present study were to investigate *B. bigemina* and *A. marginale* in cattle, sheep, goats and wild goats using indirect ELISA test in Erbil, Iraq.

### Materials and methods

Blood samples were collected from 44 cattle, 59 sheep, 70 goats and 11 wild goats in Erbil district for a period of a year 2010. Samples were selected randomly from each

animal. Information about age, breed and sex was recorded. Sera were separated by centrifugation and stored at -20 C°.

The study was conducted in north, east, south and west of Erbil district including 15 location. The distribution of location as shown in Figure 1. The north part extend Shaglawra to the Mergasur. East includes Bnaslawra, Kasnazan and Koyisnjaq. South covers Gushtapa and Makhmur area and west include Khabat, Aenkawa and Bahrka.

Thin and thick blood smears were prepared from the peripheral blood and jugular vein blood of the goat, sheep and cattle. The smears were air dried, fixed in absolute methanol and stained for 30 min in a 5% dilution of Giemsa solution in PBS, pH 7.2 or stained for 3 min by malaria kit stain. The slides were examined with oil immersion x100.

The sera of animal's samples were detected for presence of antibodies against *B. bigemina* and *A. marginale* using ELISA technique. The kits were supplemented by SVANOVIR ® Company for the two micro-organisms.

The ticks collected from the animals were put into tubes containing 70% ethanol and were examined under a stereo microscope. Morphological characterization of ticks was carried out using a stereoscopic microscope according to the keys given by (10-13). For the confirm identification the specimens were sending to the Iraq Natural History Research Center and Museum in Bagdad according to the letter No.787 in 4-10-2011.

### Results

Based on the microscopical and serological test for *B. bigemina* and *A. marginale* it have been found that 16, 6, 8 and 2 in cattle, sheep, goats and wild goats respectively were be sero-positive for *B. bigemina* and *A. marginale* antibodies as showed in Table I. In case of *B. bigemina* infection eight from 22 samples positive in serology were also positive in microscopic examination but in *A. marginale* were two from 10 samples.

The Figure 1 showed that highest prevalence of *B. bigemina* was found in cattle (27.27%) and lower value (6.77%) was detected in sheep, also the highest rates of positive prevalence *A. marginale* (9.09%) were diagnosed in cattle and wild goats while lower value (3.36%) in sheep.

Table 1: Number of infected animals by *B. bigemina* and *A. marginale* and co-infection between them.

Animal	No. of examined animals	No. of sero-positive with		Total	sero-positive co-infection between <i>B. bigemina</i> and <i>A. marginale</i> in same animals
		<i>B. bigemina</i>	<i>A. marginale</i>		
Cattle	44	12	4	16	4
Sheep	59	4	2	6	2
Goats	70	5	3	8	3
Wild goats	11	1	1	2	1
Total	184	22	10	32	10

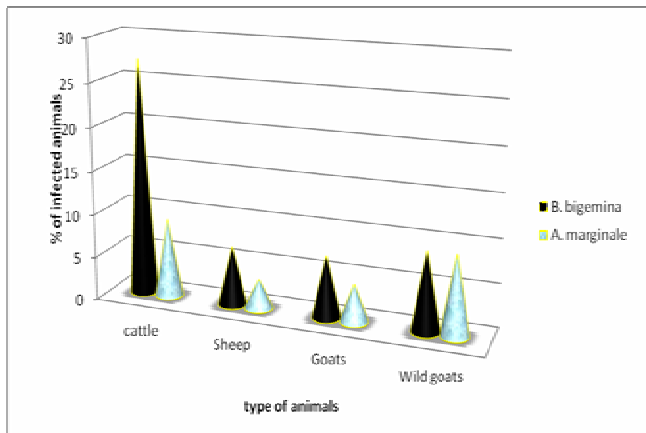


Figure 1: Percentage of infection by *B. bigemina* and *A. marginale* in domestic and wild animals.

Result of Figure 2 show that *B. bigemina* was observed more frequently in April (45.45%) than in both June and July (18.18%) than in both August and September (9.09%), *B. bigemina* could not be identified in any of the blood sera analyzed in the other time periods, also *A. marginale* observed only in April, June, July and September (30%), (40%), (20%) and (10%) respectively.

Result in Figure 3 indicated that cattle infection by *B. bigemina* peaked in April (66.66%) and *A. marginale* were peaked in June (50%).

The infection sheep by *B. bigemina* was equal separated between April, June, August and September but in the *A. marginale* only observed in April and June (Figure 4).

The results in Figure 5 showed that the samples positive for *B. bigemina* and *A. marginale* antibodies in wild and domestic goats was peaked in July.

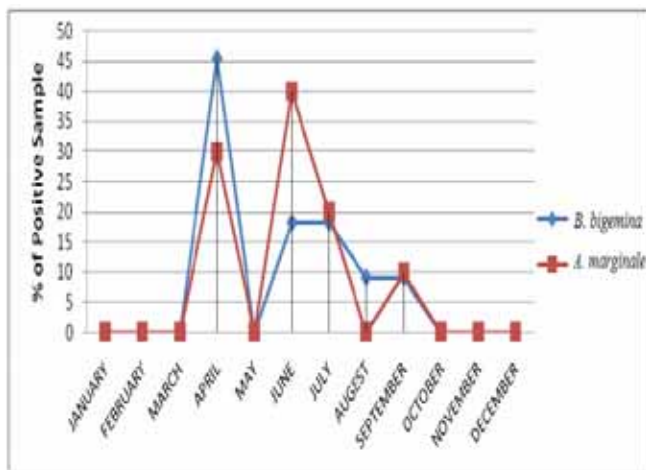


Figure 2: Seasonal dynamic infection by *B. bigemina* and *A. marginale* in domestic and wild animals.

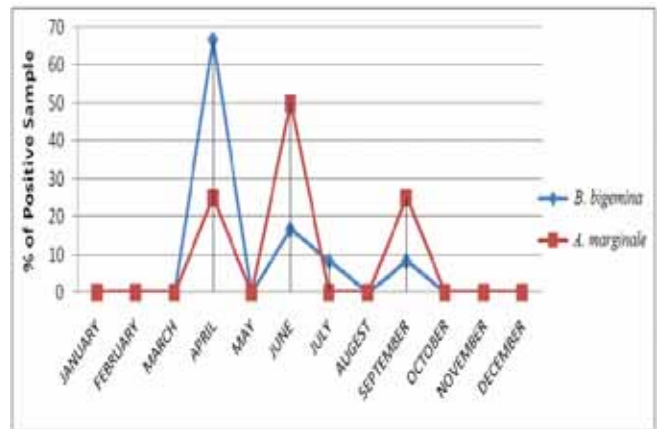


Figure 3: Seasonal dynamic infection by *B. bigemina* and *A. marginale* in cattle.

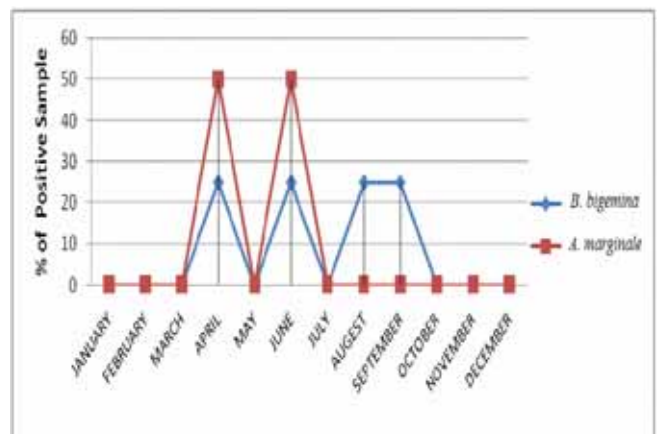


Figure 4: Seasonal dynamic infection by *B. bigemina* and *A. marginale* in sheep.

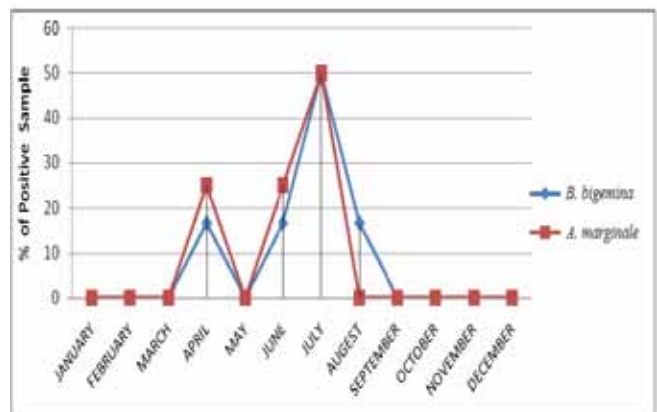


Figure 5: Seasonal dynamic infection by *B. bigemina* and *A. marginale* in wild and domestic goats.

It was found that's out of the 32 positive infections there was only 10 animals infected by *B. bigemina* and *A. marginale* in the same time (co-infection between *B. bigemina* and *A. marginale*) and which represented of (25%) cattle, (33.33%) sheep, (37.5%) goats and (50%) in wild goats as showed in Figure 6, on the other hand the highest prevalence of co-infection was in June (40%) Figure 7.

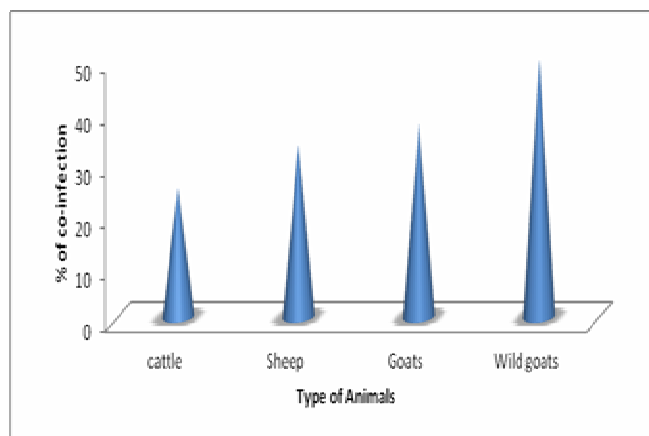


Figure 6: Percentage of co-infection between *B. bigemina* and *A. marginale* in domestic and wild animals.

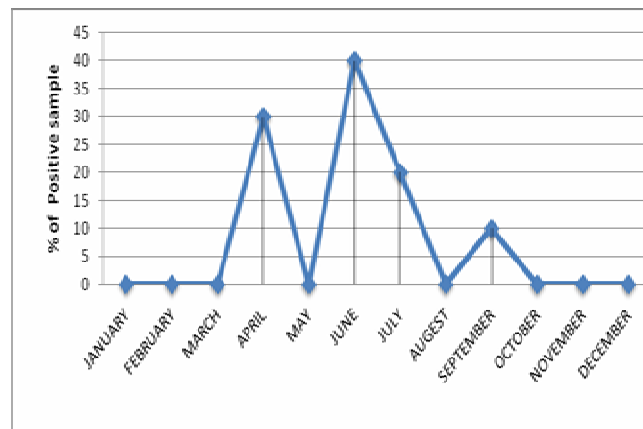


Figure 7: Co-infection between *B. bigemina* and *A. marginale* in domestic and wild animals.

In this study, 205 ticks were collected from the animals examined and the following species were detected: *Rhipicephalus sanguineus* (24.4%), *Rhipicephalus turanicus* (20%), *H. anatolicum excavatum* (19.5%), *Hyalomma anatolicum anatolicum* (12.2%), *H. marginatum marginatum* (11.2%), *H. turanicum* (6.3%), *H. detritum* (3.9%) and *Boophilus annulatus* (2.4%) as the showed in the table 2.

Table 2: Numbers of tick species and there stags collected from indigenous cattle, sheep and goats during the period from January to December 2010.

Tick species	Cattle			Sheep			Goats			General %
	♂	♀	Total	♂	♀	Total	♂	♀	Total	
<i>R. sanguineus</i>	4	4	8	11	7	18	14	10	24	24.4
<i>R. turanicus</i>	3	4	7	14	5	19	10	5	15	20
<i>H. anatolicum excavatum</i>	4	2	6	10	9	19	11	4	15	19.5
<i>H. anatolicum anatolicum</i>	1	2	3	7	3	10	8	4	12	12.2
<i>H. marginatum marginatum</i>	2	2	4	6	2	8	6	5	11	11.2
<i>H. turanicum</i>	2	0	2	2	1	3	5	3	8	6.3
<i>H. detritum</i>	0	0	0	2	1	3	3	2	5	3.9
<i>B. annulatus</i>	2	3	5	0	0	0	0	0	0	2.4
Total	18	17	35	52	28	80	57	33	90	205

## Discussion

This study was the first in Erbil province on Seroprevalence of *B. bigemina* and *A. marginale* in domestic Animals. No work has been previously done in regard to Seroprevalence of *B. bigemina* on sheep, goat and wild goats in Iraq. The low incidence of both *B. bigemina* and *A. marginale* indicates the low spread distribution of Babesiosis and Anaplasmosis in the area of study, this might be due to the geographical distribution of tick vectors transmitted these diseases and the low distribution of the

principle vector of *B. bigemina* as to reviewing. *B. bigemina* can transmitted by *Boophilus microplus*, *B. decoloratus*, *B. annulatus*, *B. geigy*, *Rhipicephalus evertsi* and *Rhipicephalus sanguineus* in Africa, Asia, Australia, Central and South America and Southern Europe (14 and 15). (16) reported that small ruminants are not severely affected by *A. marginale* and cattle show an innate resistance to *A. ovis*. The earliest survey on piroplasmosis in Erbil province were done by (17) in an FAO report, he showed that 12.5% of sheep were infected by *B. motasi*. (18) mentioned that 22.5% of cattle infected by *A.*

*marginale* in Mosul province. (19) evaluation prevalence of sheep blood parasites in 1987 in Mosul, Iraq and was declared that 36.3% of animals were theileria and babesia positive. Also (20) demonstrated that 15.42% of the native goats were infected with *Babesia ovis*, *B. motasi*, *B. foliata* and *B. taylori* in Mosul. (21) Showed that 5.6% of sheep were infected with *B. motasi* in Sulaimanyiah province. (22) Observed that a total of 704 sheep were examined, 80 (11.36%) were found infected with *A. ovis* and 19 (2.70%) with *B. motasi* in the central part of Iraq. Babesiosis in domesticated animals in Mazandaran, North of Iran was 18.13% in cows, 16.03% in sheep and 22.27 in goats (23). The antibody activity against *B. ovis* antigen was high with an overall prevalence of 41.02% in Awassi sheep in Urfa, Turkey (24). In the seroepidemiology study of *B. bigemina* in cattle in the Konya province, Turkey, samples from 770 cattle from 74 barns were examined microscopically and serologically for *B. bigemina*, based on these examinations, 15 (1.95%) cattle were found to be positive for *B. bigemina* and 331 (42.9%) cattle were diagnosed as positive for *B. bigemina* antibodies (25). According to original data from serological studies conducted in different regions of the world, seroprevalence of *B. bigemina* varies between 40% and 93% (26, 27, 28, 29 and 30).

In the presence study there was clear differences between cattle infection by *B. bigemina* and other examined animals (Table 1), this result was usual because of the *B. bigemina* considered the most important bovine causative agent worldwide, and due to cattle infection by *B. annulatus* the principle vector of *B. bigemina* in the area of the study as they showed in the table 2. (15) showed that the bovine babesiosis in Asia are *B. bovis* and *B. bigemina*. This study demonstrated the high co-infection between *B. bigemina* and *A. marginale* in all infected animal (table 1). (31) reported high mixed infections between *Anaplasma spp.* and *Babesia spp.* in infected sheep. PCR-based techniques allow detection of parasites at low parasitaemia while discriminating various species of co-infecting agents (32). TBDs can co-infect cattle causing considerable losses to the livestock industry. (33). The prevalence of infection between seasons was found to be difference (Figure 3, 4, 5 and 6) the infection by *B. bigemina* and *A. marginale* in domestic and wild animals was found between April and October. In order to determine the epidemiology of tick borne diseases, it is crucial to know the seasonal activities of the ticks. So in the present study all infection by tick and TBDs arise in spring and summer. (34) showed that the seasonal distribution of *B. ovis* antibodies peaked in spring (76% and 74.3%) in sheep and goats respectively followed by summer (75.6% and 73.7%) in sheep and goats, respectively. The highest prevalence of *Babesia spp.* was observed during spring and summer which are considered the seasons of high activity of tick vector, (35-37). Seasonally, the prevalence of *Babesia spp.* infection started

to increase in April and reached highest values in August, while a decrease was observed in September, reaching the lowest levels In February and March (38).

Many ticks were identified during the investigation which were seropositive animals in farms, and the goats showed high infection by Ixodid ticks 43.9% (table 2), *R. sanguineus* showed more frequent (22.4%) while *B. annulatus* showed lower percentage.

## Conclusion

It was found that 17.39% of domestic animal in Erbil district using of ELISA were infected by *B. bigemina* and *A. marginale*.

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