Iraqi Journal of Veterinary Sciences, Vol. 26, Supplement III, 2012 (109-114)
Proceedings of the 6th Scientific Conference, College of Veterinary Medicine, University of Mosul

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

K.A.H. Ameen[1], B.A. Abdullah[2] and R.A. Abdul-Razaq[2]

[1] Department of Plant Protection, College of Agriculture, University of Salahaddin, Erbil,
[2] Department of Biology (Microbiology), College of Science, University of Mosul, Mosul, Iraq

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®^ *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.
Available online at http://www.vetmedmosul.org/ijvs

التحري مصليا عن البابيزيا و الأنابلازما في الحيوانات الأليفة في أربيل، العراق

خالص احمد حمه أمين[1]، باسمة احمد عبد الله[2] ورياض احمد عبد الرزاق[2]

[1]كلية الزراعة، جامعة صلاح الدين، أربيل، [2]كلية العلوم، جامعة الموصل، العراق

الخلاصة

تم التحري مصليا عن *Babesia bigemina* و *Anaplasma marginale* في الماشية والأغنام والماعز الأليف والبري في محافظة اربيل للفترة من كانون الثاني ٢٠١٠ ولغاية كانون الأول ٢٠١٠، اذ جمع (١٨٤) عينة دم من (٤٤) مواشي و(٥٩) اغنام (٧٠) ماعز أليف و(١١) ماعز بري وذلك لتحضير مسحات الدم وعينات المصل التي اختبرت للتحري عن الأجسام المضادة للـ عن *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 micro-organisms, 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.

Theileriases 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 rickettisial 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 (7and 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 investigated *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 Cº.

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 Shaglawa to the Mergasur. East includes Bnaslawa, 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 *B. bigemina* and *A. marginale* in same animals
		B. bigemina	*A. marginale*		
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

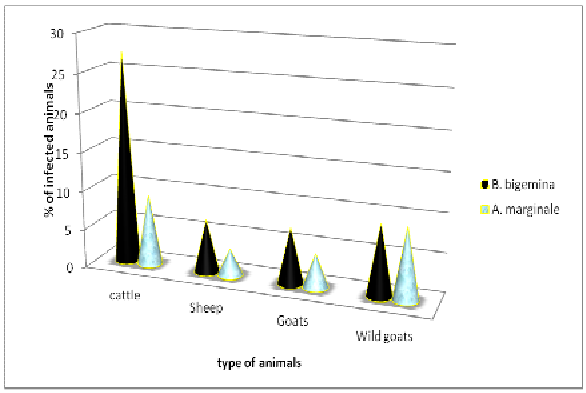

Figure 1: Percentage of infection by *B. bigemina* and *A. marginale* in domestic and wild animals.

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* pecked 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.

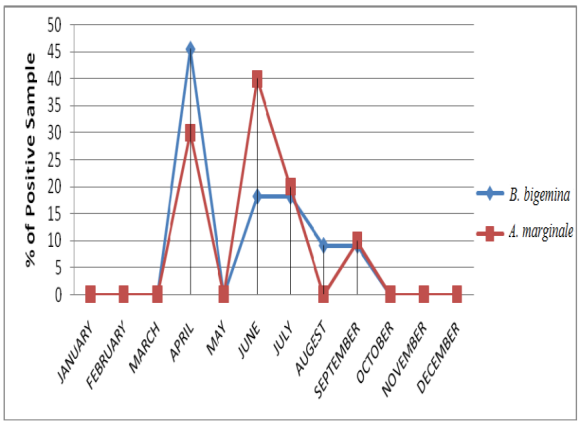

Figure 2: Seasonal dynamic infection by *B. bigemina* and *A. marginale* in domestic and wild animals.

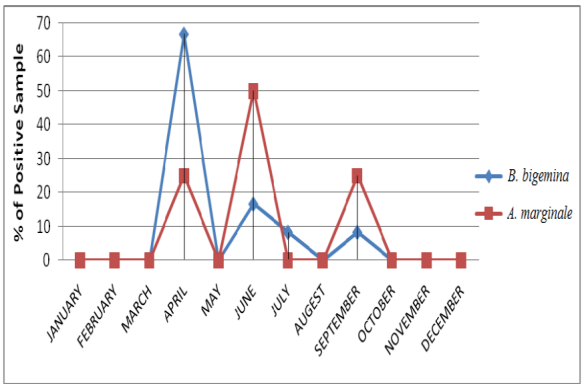

Figure 3: Seasonal dynamic infection by *B. bigemina* and *A. marginale* in cattle

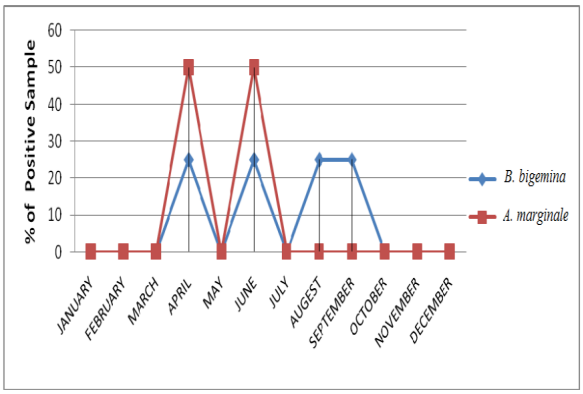

Figure 4: Seasonal dynamic infection by *B. bigemina* and *A. marginale in* sheep.

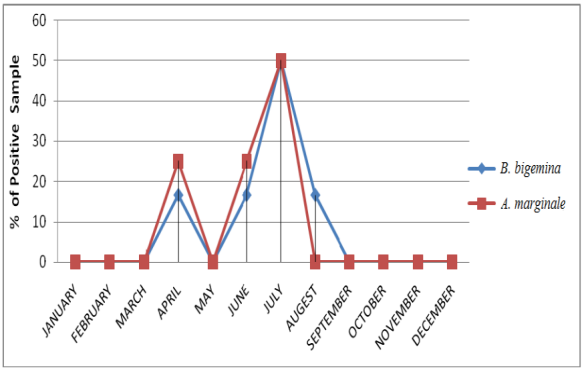

Figure 5: Seasonal dynamic infection by *B. bigemina* and *A. marginale* in wild and domestic goats.

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.

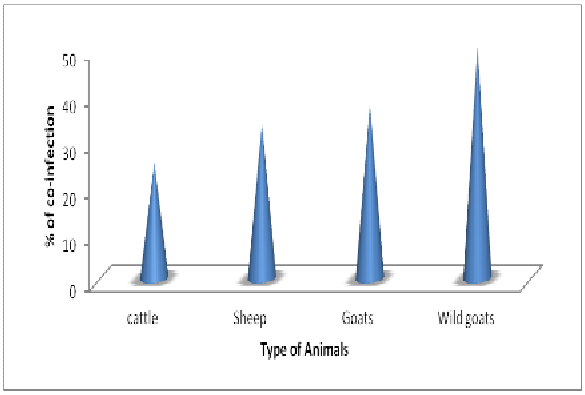

Figure 6: Percentage of co-infection between *B. bigemina* and *A. marginale* in domestic and wild animals.

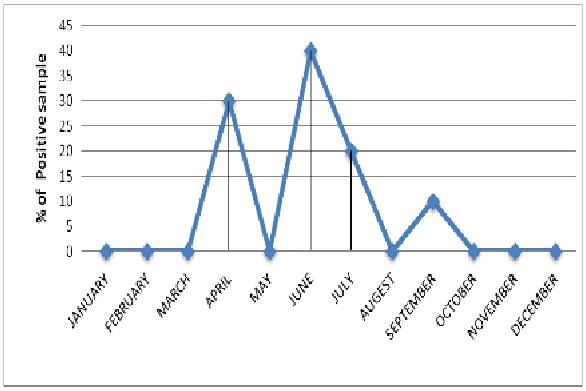

Figure 7: Co-infection between *B. bigemina* and *A. marginale* in domestic and wild animals.

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	%
R. sanguineus	4	4	8	11	7	18	14	10	24	24.4
R. turanicus	3	4	7	14	5	19	10	5	15	20
H. anatolicum excavatum	4	2	6	10	9	19	11	4	15	19.5
H. anatolicum anatolicum	1	2	3	7	3	10	8	4	12	12.2
H.marginatum marginatum	2	2	4	6	2	8	6	5	11	11.2
H. turanicum	2	0	2	2	1	3	5	3	8	6.3
H. detritum	0	0	0	2	1	3	3	2	5	3.9
B.annulatus	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. geigyi*, *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.*

References

1. EFSA (European Food Safety Authority). Scientific Opinion on Geographic distribution of Tick-borne Infections and their Vectors in Europe and the other Regions of the Mediterranean Basin1, EFSA 2010; J. 8(9):1723.
2. Minjauw B and McLeod A. Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihood of small-scale and marginal livestock owners in India and eastern and southern Africa. Research report 2003; DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK, DFID AnimalHealth Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK.
3. Hunfeld, K P, Hildebrandt and J S Gray. Babesiosis: Recent insights into an ancient disease. Int. J. for Para 2008; 38(11):1219-1237.
4. Uilenberg G. Babesia – a historical review. *Vet. Para.*2006; 138, 3–10.
5. Kocan K.M., Blouin E.F. and Barbet A.F. Anaplasmosis control. Past, present, and future, Ann. N.Y. Acad. Sci. 2000; 916, 501-509.
6. Palmer GH, Brown WC and Rurangirwa FR. Antigenic variation in the persistence and transmission of the ehrlichia Anaplasma marginale. Microbes Infect 2000; 2: 167-17.
7. Melendez RD. Future perspective on veterinary hemoparasite research in the tropic at the start of this century. Ann. N.Y. Acad. Sci. 2000; 916, 253–258.
8. Stuen S, Nevland S and Moum T. Fatal cases of tick-borne fever (TBF) in sheep caused by several 16S rRNA gene variants of *Anaplasma phagocytophilum.* Ann. N.Y. Acad. Sci. 2003; 990, 433 434.
9. Marchette N and Stiller D.The Anaplasmataceae, Bartonellaceae and Rochalimaea quintana. In:Marchette, N.J. (Ed.), Ecological Relationships and Evolution in the Rickettsiae. CRC Press, Boca Raton, Florida, USA1982; p.98-106.
10. Hoogstaal H and Kaiser MN. The Ticks (Ixodoidea) of Iraq: Keys, host and distribution. J. of Iraq Med. Prof 1958; vol.6, 2-3, June-September.
11. Hoogstaal H, Wassef HY and Buttiker.Ticks (Acarina) of Saudi Arabia Fam. Argasidae,Ixodidae 1981; Fauna of Saudi Arabia 3.
12. Walker AR, Bouattour A, Camicas JL, Estrada-Peña A., Horak IG, Latif A.A., Pegram R.G. and Preston P.M. Ticks of Domestic Animals in Africa: a Guide to Identification of Species, Bioscience Reports, 42 Comiston Drive, Edinburgh EH10 5QR, Scotland, U.K 2007.
13. Apanaskevich DA and Horak IG. The genus Hyalomma Koch, 1844: re-evaluation of the taxonomic rank of taxa comprising the *H. (Euhyalomma) marginatum* koch complex of species (Acari: Ixodidae) with redescription of all parasitic stages and notes on biology. Inter. J. of Acarol.2008; 34:1, 13 - 42.

14. Estrada-Pena A and Jongejan F. Ticks feeding on humans: a review of records on human-biting Ixodoidea with special reference to pathogen transmission. Exp. Appl. Acarol.1999; 23: 685- 715.
15. Bock R, Jackson L, De Vos A. and Jorgensen W. Babesiosis of cattle. Para. 2004; 129: S247- S69.
16. Nakamura Y, Kawazu S and Minami T. Antigen profiles of *Anaplasma ovis* and *A. mesaeterum* and cross infection trials with them and *A. marginale*. Vet. Microbiol.1993; 37: 19-30.
17. Leiper JWG. FAO report 1965; No.610 to government of Iraq on animal parasites and their control.
18. Shukir AAK. Clinical and haematological studies on chronic cases of Theileriosis and Anaplasmosis in local breed cows. Diploma thesis, College of Veterinary Medicine University of Mosul 1982.
19. Al-Hadethi H and Al-Saffar TM. Previlance of parasitic infection of sheep in northern Iraq. J. Vet. Para.1988; 2:93-95.
20. Sulaiman EG, Arslan SH, Al-Obaidi QTE and Daham E. Clinical, haematological and biochemical studies of babesiosis in native goats in Mosul. Iraqi J. of Vet. Sci.2010; 24, 1, 31-35.
21. Mustafa BH. Epidemiology study of ovine piroplasmosis (Babesiosis and Theileriosis) in Sulaimanyia Governorate, north Iraq. M.Sc. Thesis submitted of the college of Agriculture Univ. of Sulaimanyia Iraq 2006.
22. Latif B, Al-Izzi SA, Majeed MA and Sultan AS. (1987). Prevalence of piroplasmosis among sheep in the central part of Iraq. The Iraqi J. Vet. Med 1987; 11: 132-138.
23. Seyyed PZ , Behzad E and Mohammad RY. Study of the Prevalence of Babesiosis in Domesticated Animals with Suspected Signe in Mazandaran, North of Iran, During 2008. J. of Anim. and Vet. Adv 2011; 10(6):712-714.
24. Emre Z, Ali DZ, Mehmet Üand Hatice S. Seroprevalence of *Babesia ovis* in Awassi sheep in Urfa, Turkey. Turk J Vet Anim. Sci. 2001; 25, 759-762.
25. Ozlem DE and Ferda S. Seroepidemiology study of *B. bigemina* in cattle in the Konya province, Turkey: endemic status. Bull Vet Inst Pulawy.2009; 53, 645-649.
26. Applewhaite LM, Craig TM and Wagner GG. Serological prevalence of bovine babesiosis in Guyana. Tropic Anim. Health Prod1981; 13, 13-18.
27. Akınboade OA and Dipeolu OO. Comparison of blood smear and indirect antibody techniques in detection of haemoparasite infections in trade cattle in Nigeria. Vet Para.1984; 14, 95-104.
28. Woodford JD, Jones TW, Rae PF, Boid R and Bell Sakyi L. Seroepidemiological studies of bovine babesiosis on Pemba Islands, Tanzania. Vet Para.1990; 37, 175-184.
29. Guglielmone AA. Epidemiology of babesiosis and anaplasmosis in South and Central America. Vet. Para.1990; 57, 109-119.
30. Barros SL, Madruga CR, Araújo FR, Menk CF, de Almeida MA, Melo EP and Kessler RH. Serological survey of *Babesia bovis, Babesia bigemina*, and *Anaplasma marginale* antibodies in cattle from the semiarid region of the state of Bahia, Brazil, by enzymelinked immunosorbent assays. Mem. Inst Oswaldo Cruz 2005; 100, 513-517.
31. Adejinmi JO, Sadiq NA, Fashanu S O, Lasisi O T and Ekundayo S. Studies on the blood parasites of sheep in Ibadan, Nigeria. African J. of Biom.Res2004; Vol.7.
32. Shayan P and Rahbari S. Simultaneous differentiation between *Theileria* spp. and *Babesia* spp. on stained blood smear using PCR, Para. Res.2005; 97, 281-286.
33. Martin CS. Differential diagnosis of tick-borne disease and population genetic analysis of *Babesia bovis* and *Babesia bigemina*. PhD thesis, Division of Veterinary Infection and Immunity Faculty of Veterinary Medicine University of Glasgow 2009.
34. Hosein HI, Samia AA, FA Ibrahim, Abou-Elnaga TR, Gebely MA, Mona AM. Seroprevalence of *Babesia ovis* in small ruminants in Siwa Oasis, Egypt. BS. Vet. Med. J 2007; 17,1,19 24.
35. Rodriguez ON, Rodriguez P, Espaine L and Rivas A. (1989): Frequency of haemoparasites in sheep in Cuba. Revista-Cubana de Ciencias Vet.1989; 20 (1): 57-70.
36. Trifonov T and Ruseve V. Epizootiologicalstudy of piroplasmosis of cattle, sheep and its ticks vectors in Stangia region of Bulgaria. Veteriinarya Sbirka 1989; 87 (4):43-46.
37. Pipano E.Observation on the seasonal distribution of blood parasites in sheep in Israel. Israel. J. Vet. Med.1991; 46 (1): 37-38.
38. Razmi,GR.An epidemiological study on ovine babesiosis in the mashhad suburb area,province of khorasan, Iran. Vet. Para. 2002;-1