Epidemiology and Seasonal Variation of Ixodid Ticks and Piroplasmida Detection in Cattle of Basrah Province, Iraq

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Abstract

Four hundred and twenty cattle belonging to different breed and age groups were investigated for infestation by ticks during the period from October 2018 to September 2019 in Basrah governorate, Iraq . Investigated cattle were found to be infested by four species of hard ticks namely (*Hyalomma anatolicum anatolicum, Hyalomma marginatum turanicum Rhipicephalus (Boophilus) annulatus, Rhipicephalu sturanicus)* .No significant difference in infestation rate was observed according to the method of cattle raising (X^2=0.455, p=0.500),however, seasonal variation in infestation with significant difference was found , higher infestation rate reported in June (63.3%) and the lowest was in January (20%) (X^2 =76.740 ,p = 0.05). In the meantime blood smears samples from the same cattle were also examined by microscopy for hemoprotozoan pathogens .The examination revealed that those cattle are infected by *Babesia spp*. (27.14%) and *Theileria spp*.(19.52%). No, significant difference in infection rate was found between male and females, but a significant variation was seen among age groups, however, age group 1-3 years revealed a high rate of infection .Seasonal variation in the infection rates were observed in infected cattle. Higher infection rates of Babesiosis and Theileriosis reported in June (50%) and (36.7%) respectively.

Keywords: *Epidemiology, Babesia bovis, Theileria annulata , Hyalomma, Rhipicephalus, Basrah, Iraq.*

Introduction

Cattle are most important source of national income for countries .The directorate of animal wealth estimated the number of cattle in Iraq about 2.5 million in 2007. We have no specific information on the races and strains of cattle that are raised in Iraq but are believed to be most of the indigenous cattle breeds and fall within the following races: AL-Janobi cows; AL-Restaki cows, AL-Sharabi cows and AL-Karadi cows; the last two races are confined to the northern region of Iraq, in addition ,there are few numbers of the strain Holstein – Friesian introduced to improve local of dairy production. There are a number of obstacles facing the progress and development of livestock industry in Iraq, mainly diseases including ticks and tick-born disease (TBDs) which are most prevalent and exert their huge impact in tropical and sub-tropical regions [1]. TBDs cause enormous losses through mortality , morbidity, productive losses and the cost control [2,3] and their effect on the immune status of infected animal [4]. The passive impact of ticks does not only acts as vectors for pathogens ,but also causes significant effects on animals, such as lack of milk production ,weight loss, skin grafting ,and predispose animal to other bacterial and fungal diseases [1,5]. The climatic condition of Iraq is favorable for growth of tick species which is contribute to potentail occurrence of Babesiosis and Theileriosis which are caused by *Babesia spp*. and *Theileria spp.,* respectively. Routine diagnosis of babesiosis and theileriosis is performed by microscopic examination of Giemsa stained blood smears and parasite viewing as well as clinical signs in severe cases ,but in subclinical infections parasite microscopically undetectable and lead to relatively high rate of false negative diagnosis [6] .Moreover, Bilgic etal. (2013) [7] pointed out that it is difficult to differentaite between species of *Babesia* based on morphological characteristics, especially in mixed infection .If animals

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recover from infection ,along –lasting carrier status occurs in which low number of erythrocytes remain infected with parasites ,and acts as carrier or reservoir for parasites, these carrier have an important role in the transmission of the infection by ticks [8].

Materials and Methods

A - Study Area and Field Sampling

The study was accomplished in Basrah province which is located in southern part of Iraq, at a latitude of 30˚30 ´ and longitude 47˚48 ´. A total number of 420 cattle were randomly sampled during the period from October 2018 to September 2019.

Samples were collected on farms with two different methods of raising were applied, those that are graze in unimproved natural pasture and those that are kept in the pens and are hand fed and watering. All cattle without regular acaricide treatment. The sample –level variables included sample size and location (northern, central, east, west and southern areas).Cattle were categorized into age classes (<1 year old to ≥ 10 years) and divided into two categories: cattle with tick burden and no tick burden.

B - Collection of tick and Blood samples

Ticks were collected with rubbing alcohol pads surrounding the skin of cattle and removed by forceps and kept in labeling screw plastic tubes-containing ethanol. All collected ticks were examined under the stereomicroscope. Species ,sex and state of feeding were recorded.

Ticks identification was done according to Hoogostaal et al. (1981) and Shubber (2014). Some of female ticks were frozen at -20˚ for DNA extraction.

Blood samples were collected from the vena jugularis from a total of 420 cattle with a 10ml disposable syringe under aseptic precautions .About 5ml of blood was collected in tubes containing Ethylene Diamine Tetra acetic Acid (EDTA) for DNA extraction and then stored in iceboxes at 4C˚.The samples were transported to the parasitology laboratory at Basrah University ,Education College for Pure Sciences, where blood smears were prepared, and fixed by using methanol and stained by Giemsa, then examined under an oil immersion 1000x objective.

C-DNA Extraction

The DNA was extracted from Blood and tick samples using DNA extraction kit (GeneiadBiotech,Taiwan) according to the manufacture instructions. The extracted DNA were tested by Nano drop spectrophotometer (Type Implen) at wave length 260/280 nm.

D - Polymerase Chain Reaction (PCR)

For the molecular diagnosis of *T.annulata ,B.microti,B.bovis ,B.ovis* and *B.motasi* in ticks and cattle, PCR reactions were performed using the specific primers for detection *T.annulata* Cytob1(F ACTTTGGCCGTAATGTTAAAC,R CTCTGGACCAACTGTTTGG) ,312bp (Bilgic etal.,2010) *B.microti* ISSrRNA(F CTTAGTATAAGCTTTTATACAGC, R A T A G G T C A G A A A C T T G A A T G A T A C A) 238bp (Inoueetal.,2015), *B.bovi*s SSrRNA (F CTGTCGTACCGTTGGTTGAC, R CGCACGGACGGAGACCGA) , 541bp (Chaudhry et al.,2010) , *B.ovis* SSrRNA (F TGGGCAGGACCTTGGTTCTTCT, R C C G C G T A G C G C C G G C T A A A T A),549bp Aktas (2005) and *B.motasi* Rap1b(F TGCGCCTTCGAGTTGTACAAGAG, R GACGGGTTGCRTAGGCCTGAC, 565bp Niu, 2016.

The amplification protocol was as follows: initial denaturation at 95C˚ for 1 minute followed by 35 cycles of 95C˚ for 50 sec for T.annulata,40 cycles of 94C˚ for 1min. for B.microti,35cycles of 94C˚ for 30sec. for *B.bovis* and *B.motasi* and 35 cycles of 94C˚ for 1min. for *B.ovis*, annealing 35 cycles at 55C˚ for 50 sec.for *T.annulata*,40 cycles of 54 C˚ for 1min.for *B.microti* ,35cycles of 50C˚ for 30sec.for *B.bovis* ,35 cycles of 62C˚ for 1min. for *B.ovis* and 35cycles of 58C˚ for 30 sec. for *B.motasi*. Extension at 72C˚ for 1min. for *T.annulata* and 72C˚ for 90sec. for *B.microti* , 72C˚ for 45sec. for *B.bovis*, 72C˚ for 1min. for *B.ovis* and *B.motasi* with final extension at 72C˚ for 10min. for all pathogens(except for *B.bovis* at 72C˚ for 7min.) in the MiniAmp plus thermocycler .The amplification products were separated on 1.5 agarose gel stained with ethidium bromide.

E- Statistical analysis:

Chi-square test was used according to the SPSS statistical program (soft ware ,Version 23) .

Results

Out of 420 cattle examined in Basrah governorate, an overall 42.5% cattle were infested by *Hyalomma anatolicum anatolicum*,32.5% with *H.turanicum*, 23.1% with *Rhipicephalus turanicua*,21.8% with *R.(Boophilus) annulatua* and 6.2% with mixed infestation.485 specimens of ticks were collected from 160 animals that mean the intensity of infestation for each animals was 3.0ticks. Cattle raised in pens recorded the highest prevalence of ticks reaching 39.5% , while cattle grazing in pastures recorded a lower prevalence (34.9%),but the differences were insignificant (X^2 = 0.455, P=0.500). No marked differences were observed between sex or between age groups regarding the prevalence of ticks infestation .However ,the monthly prevalence of infestation in cattle, H. a. anatolicum, H. turanicum, R. turanicus and R.B.annulatus was highest in June(63.3%) and the lowest in January (20%).There were asignificant difference(X^2 =76.7,P=0.05) in monthly prevalence (Table 1).

Table (1) Monthly prevalence of ticks

Months	No.of examined cattle	No. of cattle infested with ticks	Pervalence (%)
October	25	10	40
November	25	10	36
December	30	7	23.3
January	25	5	20
February	30	8	26.7
March	25	12	48
April	40	18	45
May	50	22	44
June	30	19	63.3
July	50	27	54
August	50	22	44
September	40	20	50
Total	420	160	38.1

X^2 =76.740,P=0.05

Microscopic examination of 420 blood smears showed that 114(27.1%) and 82(19.5%) of cattle were positive for *Babesia spp*. and *Theileria spp*. respectively, according to the sex of cattle, however, there were no significant differences in the prevalence of infection.

Higher prevalence of infection with bovine babesiosis and theileriosis were recorded in cattle of 1-3 years age group 32.3%(50/155) and 22%(34/155) respectively ,while lower prevalence recorded in ≥ 10years for babesiosis 0.1(1/11) and 0%(0/11) for theileriosis. There were significant differences between age groups (X^2=19.88,P=0.001) and (X^2=24.81,P=0.00) respectively .

The seasonal variation of infection showed that the highest rate of infection were found in June ,represented by 50% for Babesiosis and 36.7% for Theileriosis ,while lower rates recorded in October 8% and 4% respectively (Table 2).There were significant differences (X^2=

88.6,P=0.00;X^2=116.69,P=0.00 and X^2= 71.5, P=0.00) respectively.

Table (2) Seasonal variation of infected Cattle with *Babesia spp.* and *Theileria spp.* in Basrah province .

Months	No.ofcattle examined	No. of cattle infected with *Babesia*	Prevalence (%)	No. of cattle infected with*Theileria*	Prevalence (%)	Mixed infection	Prevalence (%)
October	25	4	16	1	4	1	4
November	25	2	8	1	4	0	0
December	30	3	10	2	6.7	0	0
January	25	3	12	5	20	2	8
February	30	4	13.3	4	13.3	0	0
March	25	7	28	5	20	2	8
April	40	15	37.5	10	25	7	17.5
May	50	15	30	12	24	8	16
June	30	15	50	11	36.7	5	16.7
July	50	21	42	12	24	7	14
August	50	18	36	9	18	6	12
September	40	7	17.5	10	25	11	27.5
Total	420	114	27.14	82	19.52	49	11.70

X^2 = 116.669 , P= 0.000), (X^2 = 71.542 , P= 0.000),(X^2 =88.659 , P= 0.000)

Frequency of babesiosis and theileriosis infections were significantly higher in cattle with tick burden than no tick burden.

In the present study using specific primers ,it was found that *H.a.anatolicum* ticks were infected by *T. annulata, B. bovis* and *B. ovis* Fig.(1) and *Rh.(Boophilus)annulatus* were infected by *T. annulata, B. microti* and *B. bovis* Fig. (2) and the cattle had been infected by *B. microti, B. ovis, T. annulata, B. bovis* and *B. motasi* Fig. (3),(4).

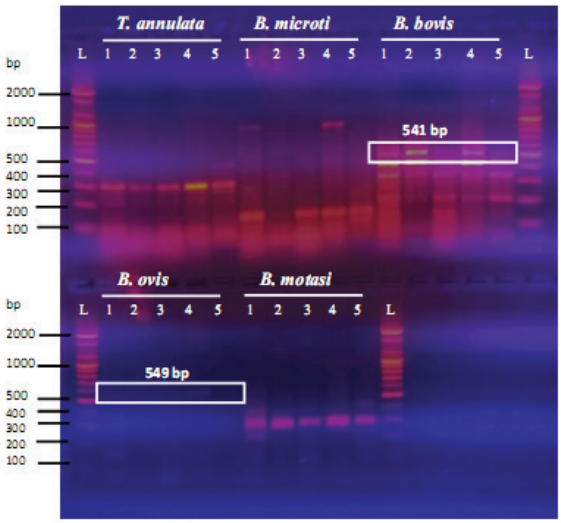

Fig.1: Agar gel electrophoresis PCR products *of H.a.anatolicum :T.annulata*(4) and *B.bovis (2) and B.ovis(4)* positive . L. (100-2000bp) represents ladder

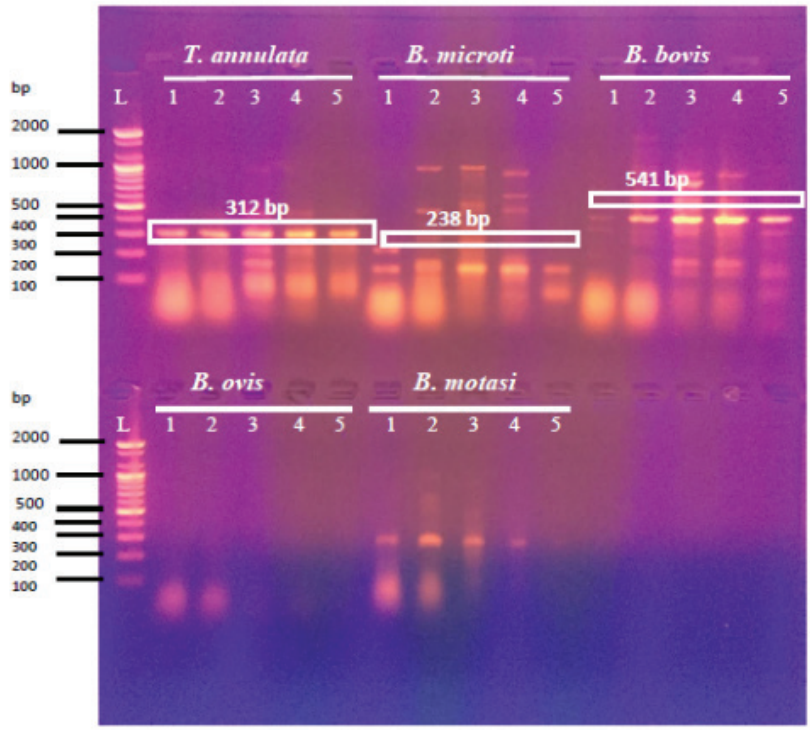

Fig.2: Agar gel electrophoresis PCR products of *Rh.(Boophilus) annulatus* , *T.annulata*(1,2,3,4,5) and *B.microti* (3) *and B.bovis* (3,4) positive . L. (100-2000bp) represents ladder

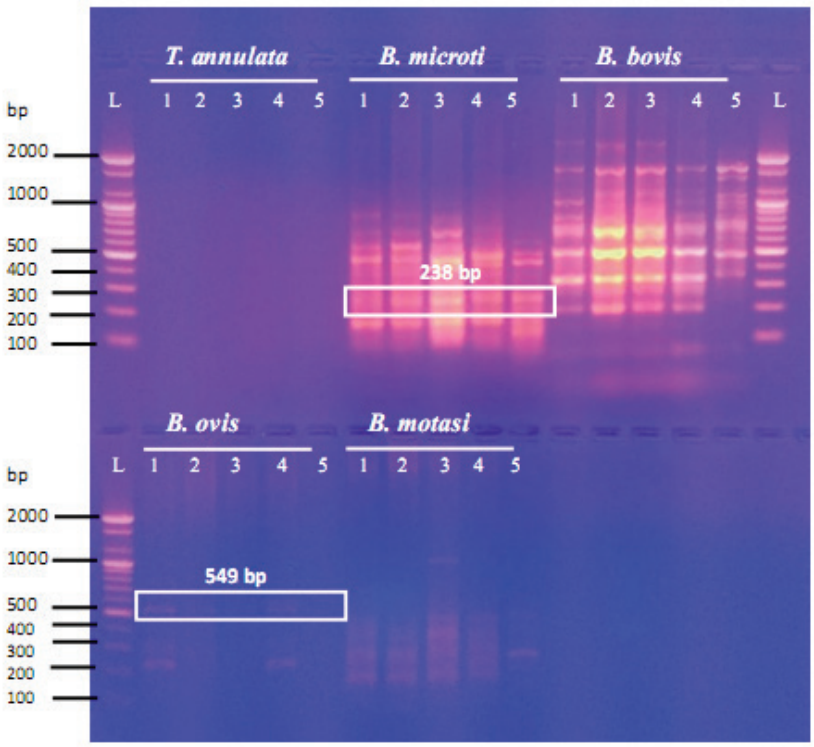

Fig.3: Agar gel electrophoresis PCR products of cattle*: B.microti* (3) *and B.ovis*(1,4) positive . L. (100-2000bp) represents ladder

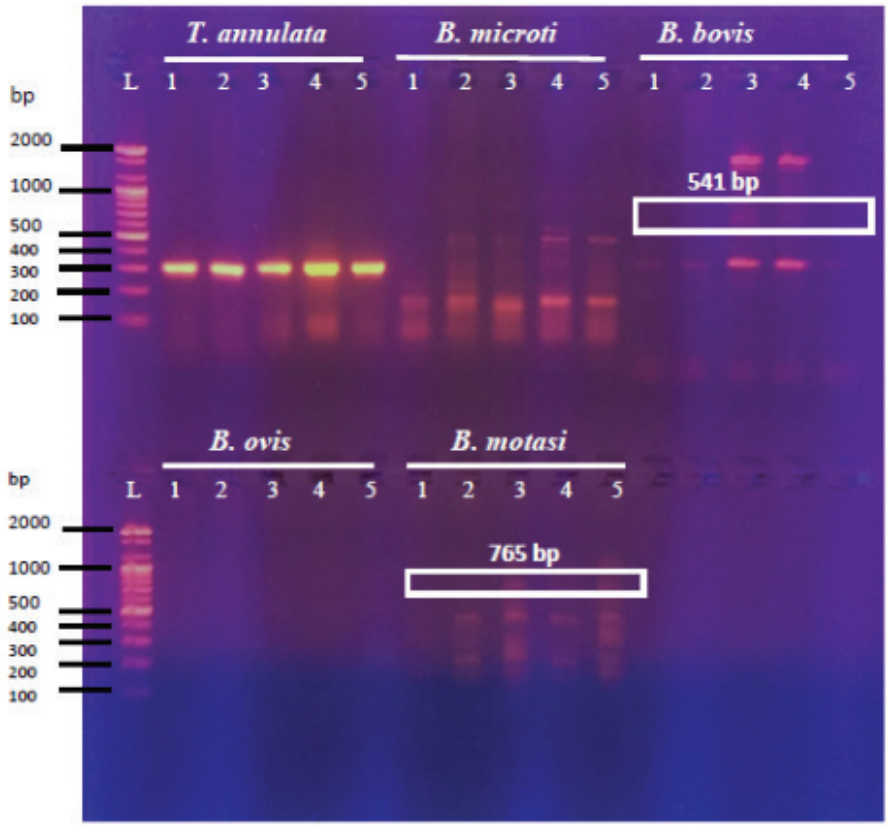

Fig.4: Agar gel electrophoresis PCR products of cattle*: T.annulata* (1,2,3,4,5) and *B.bovis* (3,4) and *B.motasi* (3) positive. L. (100-2000bp) represents ladder

Discussion

Iraq is located in the southern part of the northern temperate zone, and this location has significant impact on its climate, which is similar to the climate of the tropical region in terms of temperatures, as it is sub-tropical.

The current study provides preliminary epizotioological data on ticks and ticks borne diseases (TBDs) in south region of Iraq .However, this study provides useful information about the species of parasitoid ticks in cattle of this area. Cattle were infested with *H. a. anatolicum, H. m. turanicum, R. turanicus* and *Rh. B. annulatus*. Thus, it does not differ in terms of diversity with the study of Abdul Hussein(2006) and Muhammad(2013) in middle and southern Iraq .Infestation rate in the present study (38.1%) differs from 48.2%recorded by Tuamaet al. (2007), 54.3% of AL-Ramahi (2011) and 62% of Mohammad(2015) from other parts of Iraq. These differences in infestation prevalence may be due to the animal raising practices and using or not using of acaricides, difference in vegetation ,rainfall rate from year to year and availability of other appropriate host.

There was no significant difference in the rate of infestation of cattle raised in pens and that grazing in the pastures, although the cattle raised in the pens recorded a higher rate of infestation (39.5%).

Although variation was observed in *H.a.anatolicum* population in different seasons, the result indicates that is the predominant tick in all season in Basrah cattle .This population dynamic pattern may be attribute to the fact that hot and dry weather isconducive for the development of *H.a.anatolicum* ticks [23]

Aktas et al.(2004) [1] have referred the abundance and diversity of ticks as well as the intensity of infestation in animals when studying the epidemiology of the diseases it transmits and this is what the current study has.

The life cycles of *Babesia* and *Theileria* parasites are very similar and closely related to them in that they are transmitted by the same vector which are ticks ,but the latter differs by having a development stage in its life cycle ,which is the infection of lymphocytes before the erythrocytes are infected ,so it is possible to explain the epidemiological picture of both diseases as it is correct on the first can be correct in the second.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the College of Education for Pure Sciences and all experiments were carried out in accordance with approved guidelines.

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