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MOLECULAR DETECTION OF *BABESIA BIGEMINA* AND *BABESIA BOVIS* IN CATTLE IN BEHAIRA GOVERNORATE

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ABSTRACT

A total of 839 cattle were examined for Babesia spp. infection in Behaira Governorate, the prevalence of *Babesia bigemina* and *Babesia bovis* was 77 (9.18%) and 2 (0.24%) using Giemsa stained blood smears and the results were confirmed by PCR. Also, the results revealed that *Babesia spp.* reach its peak in summer (19.43%), and the lowest one in winter (1.05%), concerning to the locality the infection rate of *Babesia spp*. was highest in El- Dalangat (14.29%) and lowest in Abouhomos (5.2%). According to the age, the results of our study revealed that animals less than one year showed less prevalence *Babesia spp.* (5.17%), and the most prevalent was animals more than three years (10.31%).

KEYWORDS: cattle- *Babesia*- PCR- Behaira.

INTRODUCTION

Babesiosis is tick-borne disease which widespread in tropical and sub-tropical regions with high economic impact worldwide. *Babesia spp*. is a protozoan parasite transmitted mainly by ticks and able to infect erythrocytes of a wide variety of domestic and wild animals.[1] Bovine babesiosis is caused by multiple species: *Babesia bigemina, B. divergens, B. bovis*, and *B. major*. Two species, *B. bigemina* and *B. bovis*, have a considerable impact on cattle health and productivity in tropical and subtropical countries.[2]

Subclinical babesiosis leads to conversion of the affected livestock to chronic carriers and in turn sources of infection for tick vectors, and cause natural transmission of the disease. Therefore, latent infection is the target in the epidemiology of the diseases.[3] Polymerase chain reaction (PCR) is more sensitive and specific technique offers an alternative approach for the detection of babesiosis.[4] This study is aimed to determine of the prevalence, epidemiology and molecular detection of *Babesia* spp. which infect cattle in Behaira governorate.

MATERIAL AND METHODS

Animals and study area

A total of 839 cattle in five localities in Behaira governorate; which represent Behaira governorate. Whereas Itay- Elbaroud was located in the South of the governorate, Shobrakheit from the East, El-Dalangat and Abo-Elmatameir from the West, and Abouhomos from the North.

2- Collection of the blood samples Collection of the blood smears from ear vein of each examined animal (three slides/ animal) for the detection of *Babesia* spp. microscopically and 5 ml blood samples from jugular vein on anticoagulant tubes containing EDTA and stored at -20 °C for DNA extraction.

3-Preparation of blood smear and microscopic examination

Blood smears were prepared according to.[5] Microscopical identification of *Babesia spp.* according to keys of.[6, 7]

DNA extraction and PCR amplification.

Extraction of *Babesia* spp. genomic DNA from 200 ml of EDTA treated whole blood was performed by using (QIA amp DNA mini Kit Qiagene kits) and according to company manufacture. Positive control samples representing *Babesia spp.* were obtained from positive clinical cases from the examined cattle. All DNA extracted samples stored at -20 C° up to use.

PCR reaction.

Specific primers targeting *B. bovis* spherical body protein-4 (SBP-4) and *B. bigemina* rhoptry-associated protein-1 (RAP-1a) to amplify the respective genes by using the described PCRs as the following, each PCR and nPCR reaction was performed in a total 20 µl volume containing 3µl of DNA template, 10µl Master mix (Intron Biotechnology Company), 1 µl (10 pmol) of each primers (Table1) and 5µl of water. The thermo cycling conditions for PCR amplification were as follows: initial denaturing at 95°C for 5 min followed by 35 cycles (denaturing at 94°C for 1 min, annealing 53°C for 90 seconds and extension at 72 °C for 1 min) and final extension for 10 min at 72°C for *Babesia bovis* and *Babesia bigemina*. Also other primer targeting *B. bigemina (Theileria equi* 18S rRNA, table (1) were used to amplify the respective genes by using the described PCRs as following conditions,: initial denaturing at 95°C for 5 min followed by 40 cycles (denaturing at 96°C for 1 min, annealing 60 °C for 1 min and extension at 72 °C for 1 min) and final extension for 10 min at 72°C for *Babesia spp.* Electrophoresis of 5 µl from PCR and nPCR products examined under UV and photographed.

Table (1): The primers, reference and product size of different primers used in the PCR assays

Pathogen Target gene	Assay	Oligonucleotide sequences (5' > 3')	Product size (bp)	Reference
B. bigemina RAP-1a	PCR	F: GAGTCTGCCAAATCCTTAC	879	Terkawi et al., (2011)
		R: TCCTCTACAGCTGCTTCG		
	nPCR	F: AGCTTGCTTTCACAACTCGCC	412	
		R: TTGGTGCTTTGACCGACGACAT		
B. bovis SBP-4	PCR	F: AGTTGTTGGAGGAGGCTAAT	907	Terkawi et al., (2011)
		R: TCCTTCTCGGCGTCCTTTTC		
T.equi 18S rRNA	PCR	(BecUF2) F: TCGAAGACGATCAGATACCGTCG	392	Alhassan et al., (2005)
		(Equi-R) R: TGCCTTAAACTTCCTTGCGAT		

RESULTS

Morphology of *Babesia* spp.

The results of the Giemsa stained blood smears examination showed the presence of different forms of both *Babesia bigemina and B.bovis* within infected red blood cells as single pear shape, double pyriform, and double irregular forms and ring forms as plate (1, 2).

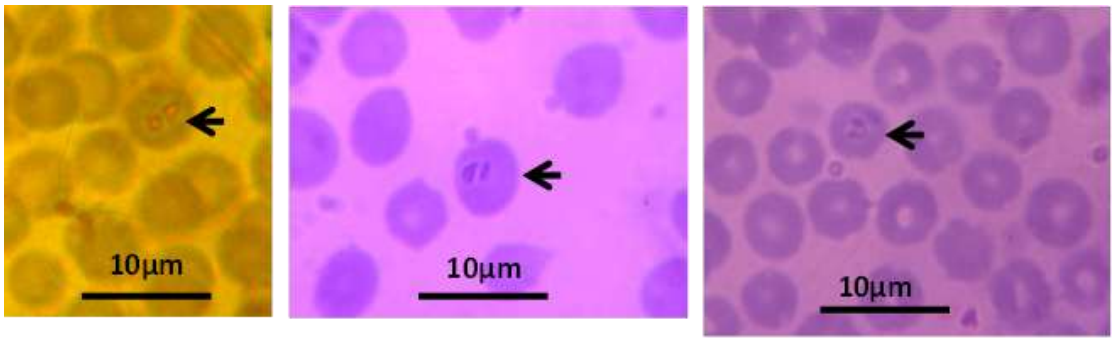


Plate (1): Blood smears from cattle showing *Babesia bigemina* (arrows), Giemsa stain, x100.

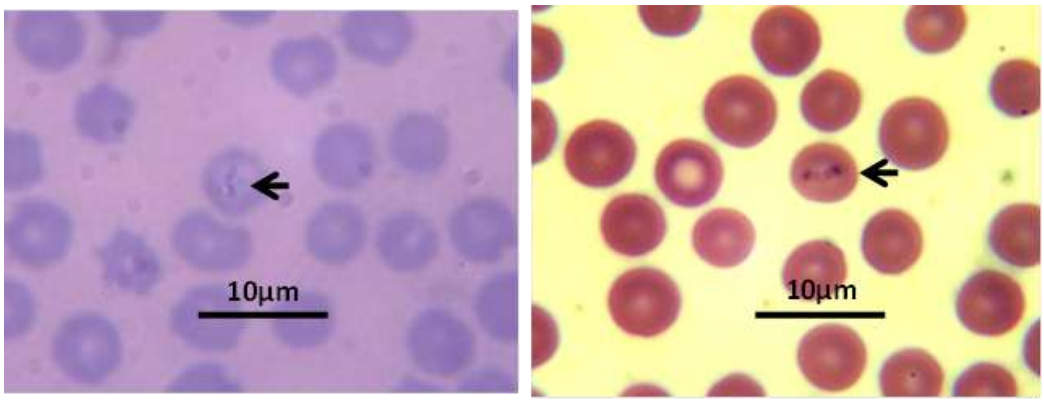


Plate (2): Blood smears from cattle showing *B. bovis* (arrows), Giemsa stain, x100.

Prevalence of Babesia spp. in cattle in Behaira Governorate.

This study was conducted on 839 cattle of different ages, foreign breeds and sexes in different five localities in Behaira Governorate.

were examined for the detection of piroplasmids, the results of Giemsa stained blood smears from cattle revealed that 77, 2 out of 839 smears showing different stages of *Babesia bigemina* and *B. bovis* representing 9.18% and 0.24% respectively (as table 2).

Table (2): The incidence of *Babesia* spp. in cattle in Behaira Governorate according to blood smears examinations.

No. of examined samples	*+ve Babesia bigemina.*		*+ve Babesia bovis*		*Total +ve Babesia spp.*	
	No.	%	No.	%	No.	%
839	77	9.18	2	0.24	79	9.42

Also *Babesia* infection reached its peak in July (26.39%) and no infection was recorded in January

Also, the results revealed that *Babesia spp.* reach its peak in summer (19.43%), followed by autumn (9.91%) then Spring (6.34%) and the lowest one in Winter (1.05%) (table 3).

Table (3): The seasonal prevalence of Babesia spp. among cattle in Behaira Governorate.

Season	No. of examined animals	*+ve Total Babesia spp.*		*+ve Babesia bigemina*		*+ve Babesia bovis*	
		+ve No.	*%*	*No.*	*%*	*No.*	*%*
Spring	205	13	6.34	13	6.34	-	-
Summer	211	41	19.43	40	18.96	1	0.47
Autumn	232	23	9.91	22	9.48	1	0.43
Winter	191	2	1.05	2	1.05	-	-
Total	839	79	9.42	77	9.18	2	0.24

Concerning to the locality the infection rate of *Babesia spp*. was highest in El- Dalangat (14.29%) and lowest in Abouhomos (5.2%).

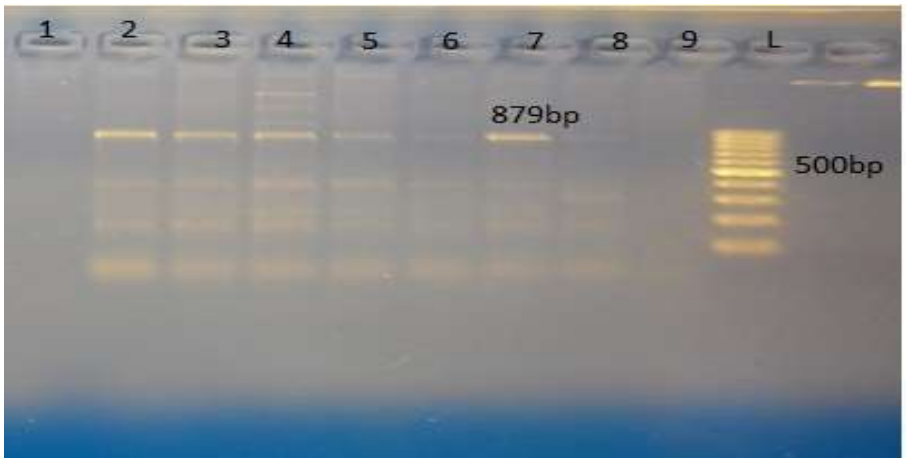
According to the age, the results of our study revealed that animals less than one year showed less prevalence of *Babesia spp.* as (5.17%), then animals 1-3 years (5.88%) and the most prevalent was animals more than three years (10.31%) (Table 4).

Table (4): The incidence of *Babesia* spp. in cattle according to microscopical examinations of Giemsa stained smears in different ages in Behaira Governorate.

Age	No. of the exam. Animals	*Babesia spp.*	
		No.	%
<one year	58	3	5.17
1-3 year	102	6	5.88
> 3 years	679	70	10.31
Total	839	79	9.42

PCR detections of *Babesia* spp. parasite.

The results of PCR and nPCR technique confirmed the diagnosis of *Babesia* spp. based upon microscopical examination of blood smears.The blood smears of positive cases was all positive by PCR.Some of blood smears negative cases are positive by PCR. The results of PCR using specific primers as, *B. bigemina* RAP-1a and *T. equi* 18S rRNA for *B. bigemina* revealed positive band at 879-bp, 412-bp and 392-bp respectively (Figures 1, 2, 3). for *B. bovis* SBP-4 give it`s expected band at 907-bp as Fig. (4).



(Fig. 1) Ethidium bromide stained agarose gel of PCR amplified fragments for *B. bigemina* at 879-bp (lane L= ladder, lane 1, 9 negative blood samples, lane 2,3,4,5,6,7,8 positive blood samples.

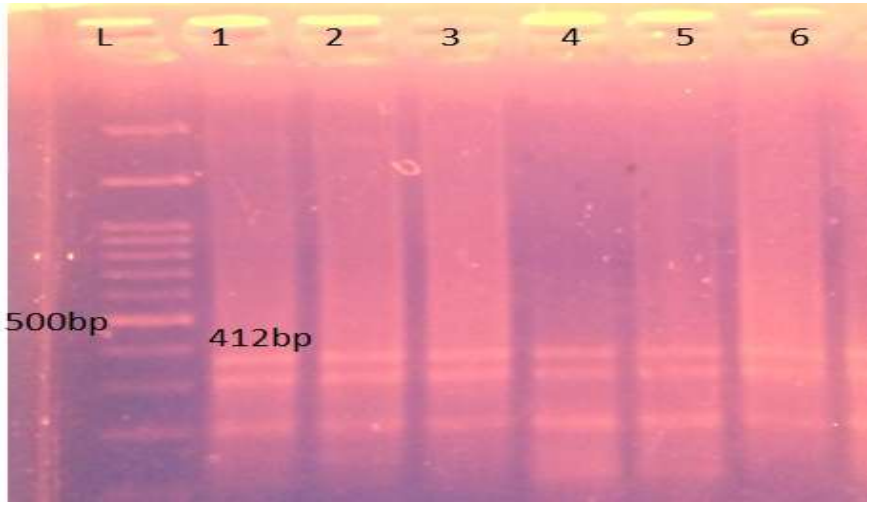


Fig. (2): Ethidium bromide stained agarose gel of nPCR amplified fragments for *B. bigemina* at 412-bp (lane L= ladder, lane 1, 2, 3, 4, 5, 6 positive blood samples.

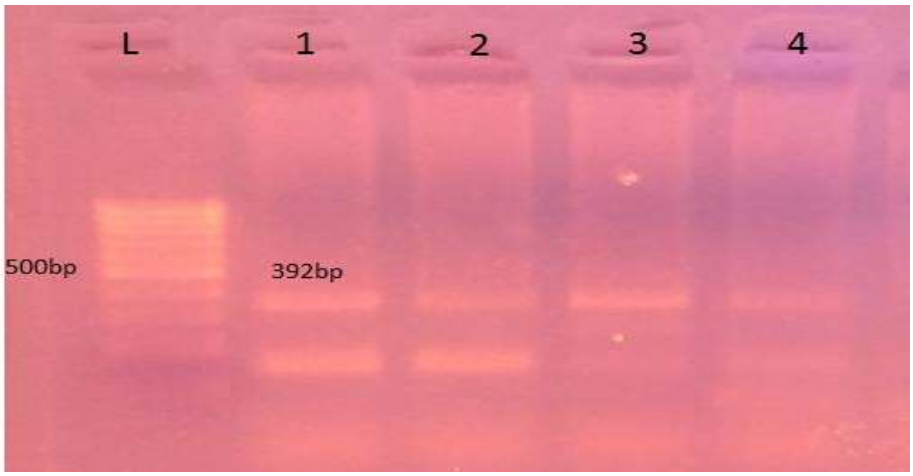


Fig. (3): Ethidium bromide stained agarose gel of PCR amplified fragments for *B. bigemina* at 392-bp (lane L= ladder, lane 1, 2, 3, 4 positive blood samples.

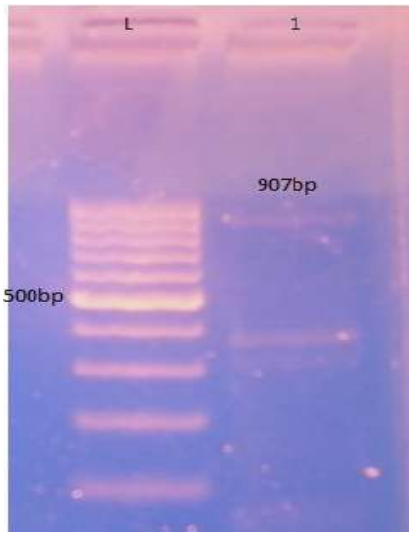


Fig. (4): Ethidium bromide stained agarose gel of PCR amplified fragments for *B. bovis* at 907-bp (lane L= ladder, lane 1 positive blood samples).

DISCUSSION

The results of our study revealed that the incidence of *Babesia spp*. was (9.42%) in agreement with many authors extensively studied *Babesia spp*. in different regions of Egypt as[8] and[9] in Assuit,[10] in Sharkia,[11] in Qalyobia,[3] in Menofia and[12] in Behaira and Fayoum. In other countries,[13] in South Italy,[14] in Pakistan and[15] in Nigeria.

While disagree with[16] in Suez Canal,[17] in Egypt,[18] in Egypt,[19] in Qalyobia Governorat,[20] recorded a higher prevalence using PCR followed by nPCR, whereas[21] in Egypt recorded higher prevalence (13.8%) by microscopic examination.

Furthermore, this study showed that the incidence of *B. bovis* (0.24%) which could be explained by the resistance of cattle leading to presence of stable disease situation. Subsequently, if cattle are latently infected, diagnosis will be difficult by blood smears. These results agree with the results obtained by[22] in Spain and in contrast with[21] 15% in Egypt using PCR assay, those may be due to use of molecular techniques which are more sensitive than stained blood smears or their studies were applied in different areas.

In the present study, the lower incidence of *B. bovis* (0.24%) than *B. bigemina* (9.18%) could be because of *B. bovis* parasites usually become infective within 2-3 days after larval ticks attach, and can be transmitted only by larvae. In *Rhipicephalus (Boophilus) microplus*, *B. bovis* does not persist after the larval stage. In contrast, *B. bigemina* matures in approximately 9 days after a larval tick attaches, and it can be transmitted by both nymphs and adults stages.[23] Therefore, under field conditions, the rate of tick transmission is generally higher for *B. bigemina* than *B. bovis*.[24]
According to the seasonal incidence of *Babesia spp*. the obtained results showed that the higher rate of infection was recorded in Summer and the lowest season was in Winter in agreement with many authors like[9] in Assuit,[10] in Sharkia,[6] in Fayoum,[25] in Giza, Beni Suef and Fayoum,[14] in Pakistan and[26] in india.

On the other hand,[24] in Kafr EL-Sheikh estimated that the higher rate of incidence of *Babesia spp*. was in autumn.

In our opinion, the increased incidence of *Babesia spp*. in Summer may be due to the highest prevalence of *Boophilus spp*. was in Summer also it could be due to decrease animal's immunity during this season due to decrease green fodders which may lead to some metabolic disorders while increase incidence of *Babesia* in Autumn than Spring was due to increase activity of tick in this season which recorded in our study.

While the infection rate of *Babesia spp*. showed the highest in El- Dalangat (14.29%) and lowest in Abouhomos (5.2%). The differences in infection rates may be due to the geographic situation of each area with the relation to ecology of tick. The most prevalent was animals more than three years which in agreement with[24] in Gharbia and partially agreed with[27] in Behaira.

The infection rate was low among the young animals may be in our opinion due to having innate resistance enhanced by maternal antibodies. This resistance declined gradually leaving the animal with a high susceptibility to disease.

Diagnosis based upon PCR is more sensitive than microscopic examination especially in the chronic infection where the presence of piroplasms in peripheral blood is scarce or non-consistent and combination of the microscopical diagnosis and molecular diagnosis is much more accurate which in agreement with[3] and[21] in Egypt. From this study; we recommend to make prophylactic treatment of cattle especially in summer season.

Conflict of interest is NIL.

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