ejpmr, 2019,6(4), 88-91

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EPIDEMIOLOGICAL PREVALENCE OF *THEILERIA SPP.* INFECTED CATTLE IN BEHAIRA DISTRICT

Nasr M. El-Bahy [1], Soad M. Menshawy[2], Wael M. Goda[1], Sherif M. Nasr[3], Eman K. Bazh[4*] and Abdelrahman A. Abou-Rwash[2]

[1]Department of Parasitology, Faculty of Veterinary Medicine, Sadat City University, Sadat, Egypt.
[2]Department of Pathology and Parasitology, Faculty of Veterinary Medicine, Damanhour University, Egypt.
[3]Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Damanhour University, Faculty of Veterinary Medicine, Egypt
[4]Department of Parasitology, Menoufia University, Faculty of Veterinary Medicine, 32511 Egypt.

***Corresponding Author: Dr. Eman K. Bazh**

Department of Parasitology, Menoufia University, Faculty of Veterinary Medicine, 32511 Egypt.

Article Received on 30/01/2019 **Article Revised on 20/02/2019** **Article Accepted on 13/03/2019**

ABSTRACT

A survey for *theileria spp* infection was conducted in five localities in Behaira district, Egypt. Of 839 cattle was surveyed and found 93 (11.08%) were positive for *Theileria spp* using Giemsa stained blood smears and the results were confirmed by PCR. *Theileria spp.* reach its peak in Autumn (15.52%), and the lowest one in Winter (3.14%), According to the age, the results of our study revealed that animals less than one year showed less prevalence and the most prevalent was animals more than three years, concerning to the locality, the infection rate of was highest in Abouhomos and lowest in Abo-ELmatameir.

KEYWORDS: *Cattle, Theileria, PCR, Behaira, Egypt.*

1- INTRODUCTION

Bovine piroplasmids are endemic in Egypt and widespread in other regions of the world including the Mediterranean Basin.[1] Bovine *Theileria* species cause severe and mild infections in their hosts.[2] Two of them, *T. annulata* and *T. parva*, cause high mortality and morbidity in cattle, commonly known as tropical theileriosis and East Coast fever, respectively. In Egypt, large number of cattle is infected with subclinical piroplasmosis.[3] Polymerase chain reaction (PCR) is more sensitive and specific technique offers an alternative approach for the detection of babesiosis.[4] The present study aimed to clarify the prevalence and epidemiology of *Theileria spp.* which infect cattle in Behaira district.

2- MATERIALS AND METHODS

2.1- Animals and samples

Blood samples from cattle were collected from five localities in Behaira district. Collection of the blood samples from ear vein of each examined animal for detection of *Theileria spp.* microscopically and 5 ml blood samples from jugular vein on anticoagulant tubes containing EDTA and stored at -20 °C for DNA extraction.-Preparation of blood smear and microscopic examination according to.[5]

2.2- DNA extraction and PCR amplification

Extraction of *Theileria* spp. genomic DNA using (QIA amp DNA mini Kit Qiagene kits) and according to company manufacture. Positive control samples representing *Theileria 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 18S rRNA genes were used to amplify the respective genes by using the described PCRs as the following, each PCR 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 as Table (1) 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 55 °C for 1 min and extension at 72 °C for 1 min) and final extension for 10 min at 72°C for. A nested PCR was done using 2 µl of DNA template obtained from the first PCR amplification. 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
*Theileria spp.*18S rRNA	PCR	F: GAAACGGCTACCACATCT	778	[21]
		R: AGTTTCCCCGTGTTGAGT		
	nPCR	F: TTAAACCTCTTCCAGAGT	581	
		R: TCAGCCTTGCGACCATAC		

3 RESULTS

1- Prevalence of *Theileria spp.*

The results revealed that 93 (11.08%) out of 839 stained smears showing different stages of *Theileria spp. Theileria spp*. infection increased in September (26.32%) and no infection in January, while infection rate in Autumn was increased (15.52%), followed by Spring (13.66%) then Summer (10.9%) and the lowest one was in Winter (3.14%) as table (2).

According to the age, the results of our study revealed that animals less than one recorded no infection then animals 1-3 years (3.92 %) and the most prevalent was animals more than three years (13.11%) as table (3).

Concerning the locality, the infection rate of *Theileria spp*. showed its highest in Abouhomos (17.92%) and the lowest in Abo-Elmatameir (8.24%) as table (4).

Table (2): The seasonal prevalence of *Theileria spp.* among cattle in Behaira Governorate.

Season	No. of examined animals	*Theileria spp.*	
		+ve No.	*%*
Spring	205	28	13.66
Summer	211	23	10.9
Autumn	232	36	15.52
Winter	191	6	3.14
Total	839	93	11.08

Table (3): The incidence of *Theileria spp.* in cattle according to microscopical examinations of Giemsa stained smears in different ages in Behaira Governorate.

Age	No. of the exam. animals	*Theileria spp.*	
		No.	%
<one year	58	0	0.0
1-3 year	102	4	3.92
> 3 years	679	89	13.11
Total	839	93	11.08

Table (4): The infection rate of *Theileria spp.* in cattle concerning to localities in Behaira Governorate.

Location	No. of exam. animals	*Theileria spp.*	
		+ve No.	%
El-Dalangat	161	16	9.94
Abo-ELmatameir	170	14	8.24
Itay- Elbaroud	166	16	9.64
Shubrakheit	169	16	9.47
Abouhomos	173	31	17.92
Total	839	93	11.08

2- Morphology of *Theileria* spp. in Giemsa`s stained blood smears.

Morphology of *Theileria spp.*, detected inside peripheral blood lymphocytes in the form of macroschizont containing large chromatin granules and microschizont which containing small chromatin granules (Fig.1)

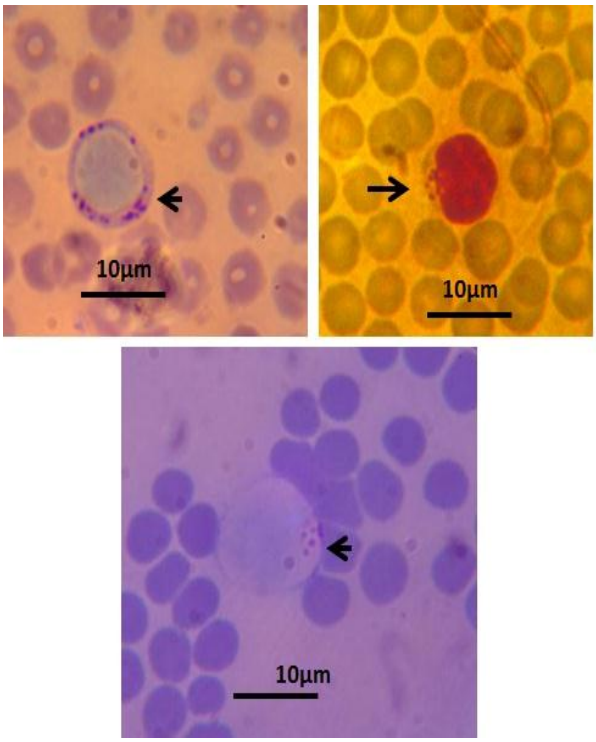

Fig. (1): Blood smears from cattle showing different macroshizont and microshizonts of *Theileria spp*. in lymphocytes (arrows), Giemsa stain, x100.

3- DNA extraction and PCR amplification of blood samples.

The results of PCR and nPCR technique confirmed the diagnosis of *Theileria* spp. based upon microscopical examination of blood smears.

The results of PCR using 18S rRNA primers for *Theileria* spp. revealed positive band at 778 and 581-bp as Fig. (2, 3).

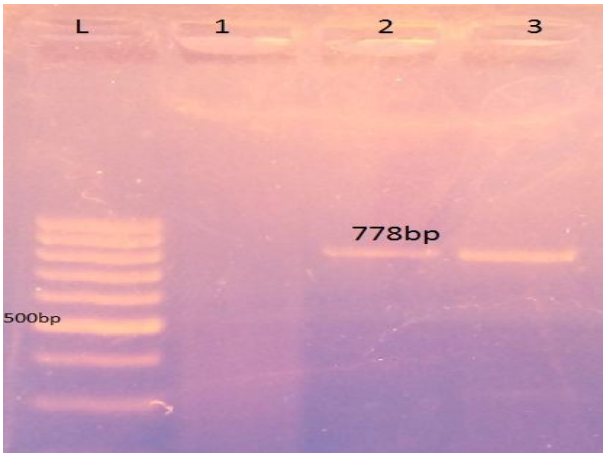

Fig. (2): Ethidium bromide stained agarose gel of PCR amplified fragments for *Theileria spp.* at 778-bp (lane L= ladder, lane 1 negative blood samples, lane 2, 3 positive blood samples).

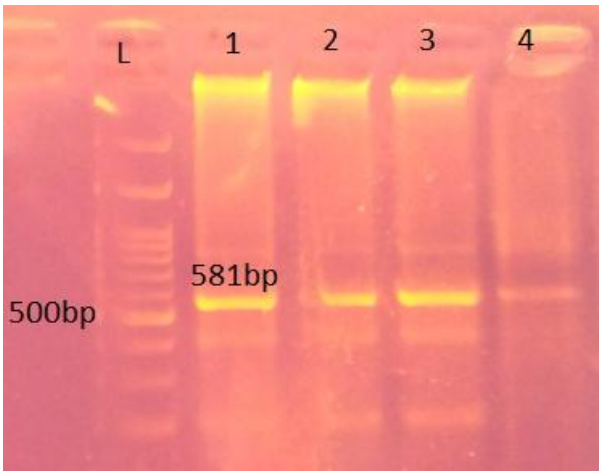

Fig. (3): Ethidium bromide stained agarose gel of nPCR amplified fragments for*Theileria spp.* at 581-bp (lane L= ladder, lane 1,2,3,4 positive blood samples.

4 DISCUSSION

The incidence of *Theileria spp.* was (11.08%) with regard to Egyptian studies, the result partially agreed with[6] was 10% in Assuit,[1] was 13% and[7] was 16.05% in Menofia, also in other countries[8] was 14.47% in Iran and[9] was 9.35% in India. Many authors opposed these results such as,[10] in Port Said in Delta region who recorded a high prevalence of theileriosis (76.5% and 33.8% respectively), and[11] in Sharkia who recorded low prevalence (1.8%).

The variations in incidence of theileriosis may be due to the differences of climatic conditions of the area of the study, immunological status of animals, different ages, breeds and sexes. Also theileriosis detectable in cattle with no signs of infection but maintained as a persistant sub-clinical state in the carrier cattle[12] in Iran.

Also, in our opinion, low incidence of theileriosis may be due to author applied his study on native breed cattle which have innate resistance against theileriosis and if it was taken infection, it could be cause less severe damage and remain carrier. Moreover, the transfer of tiny amounts of blood between animals when vaccination by sharing of syrings may induce the immunological responces.

In the present study, the infection rate of *Theileria spp.* increase in Autumn and showed the lowest rate in Winter. It was found to be in agreement with[22] in Qalyobia governorate,[13] in Saudi Arabia,[23, 14] in India.

Whereas disagree with[15] in Giza, Beni Suef, El-Minyia and Fayoum governorates who found the highest incidence of *Theileria spp.* in Summer and the lowest in Winter,[16] in Gharbia governorate who recorded that the high incidence in Winter and the lowest one in Summer and[9] in india.

The seasonal variations in the incidence of *Theileria spp.* infection could be explained according to vector. In Egypt, it is an open marketing which enable animals to transport from other surrounding infected governorates in which *Hyalomma spp.* present as reported by[11] in Sharkia,[17] in Ismailia and Giza,[18] in Egypt and[3] in Giza Governorate.

The most prevalent was animals more than three years which in agreement with[24] in Assuit,[16] in Gharbia and partially agreed with[19] 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.

Also the infection rate of *Theileria spp.* showed its highest in Abouhomos (17.92%) and the lowest in Abo-Elmatameir (8.24%), The differences in infection rates may be due to the geographic situation of each area with the relation to ecology of tick; also these localities may be bordered ones to the infected surrounding provinces.

Diagnosis based upon PCR is more sensitive than microscopic examination especially in the chronic infection which in agreement with[7, 20] in Egypt. From this study; we recommend to make prophylactic treatment of cattle especially in autumn season.

5 REFERENCES

1. Ibrahim, Ak, EL Behairy AM, Mahran KA, Awad WS. Clinical and laboratory diagnosis of piroplasmids in naturally infected cattle in Egypt. J. Egypt. Vet. Med. Assoc, 2009; 69(2): 105-203.
2. Safieldin M, Abdel Gadir A, Elmalik KH. Factors affecting seasonal prevalence of blood parasites in dairy cattle in Omdur-man locality, Sudan. J Cell Anim Biol., 2011; 5(1): 17–19.
3. Adham FK, AbdEl Samie EM, Gabre RM, El-Hussein H. Detection of tick blood parasites in Egypt using PCR assay I- *Babesia bovis* and

Babesia bigemina. Parasitol Res., 2009; 105: 721-730.
4. AbouLaila M, Yokoyama N, Igarashi I. Development and evaluation of two nested PCR assays for the detection of *Babesia bovis* from cattle blood. Vet Parasitol, 2010; 172: 65-70.
5. Kruse GOW, Pritchard MH. The collection and preservation of animal parasites. HW. MI., University of Nebraska Press, Lincolin, USA, 1982.
6. Sakla, AA. Studies on ticks in Assiut Governorate, with special reference to their role in transmission of parasitic diseases. Ph. D. Thesis, Fac. Vet. Med., Assiut Univ., Egypt, 1975.
7. Nayel M, El-Dakhly KM, Aboulaila M, Elsify A, Hassan H, Ibrahim E, Salama, A, Yanai T. The use of different diagnostic tools for Babesia and Theileria parasites in cattle in Menofia, Egypt. Parasitol Res., 2012; DOI 10.1007/s00436-012-2926-6.
8. Dehkordi FS, Parsaei P, Saberian S, Moshkelani S, Hajshafie, P, Hoseini SR, Babaei M, Ghorbani MN. Prevalence study of Theileria annulata by comparison of four diagnostic techniques in Southwest Iran. Bulg. J. Vet. Med., 2012; 15(2): 123−130.
9. Kumar V, Kaur P, Wadhawan VM, Pal H, Sharma H, Kumar P. Theileriosis in cattle : Prevalence and seasonal incidence in Jalandhar district of Punjab (India). International Journal of Recent Scientific Research, 2015; 6(3): 2998-2999.
10. Ghoneim AM, El-Fayomy Asmaa O. Targeting tams-1 gene results in underestimation of Theileria annulata infection in diseased cattle in Egypt. Acta Parasitologica, 2014; 59(1): 85–90. ISSN 1230-2821.
11. Ahmed AB. some biological studies on blood parasites of farm animals. Ph.D. Thesis. Fac. Ve. Med. Zagazig Univ, 1980.
12. Noaman V. A molecular study on Theileria and Babesia in cattle from Isfahan Province, Central Iran. J. Parasit. Dis., 2013; 37(2): 208–210.
13. El-Metenawy TM. Prevalence of blood parasites among cattle at the central area of Saudi Arabia. Veterinary Parasitology, 2000; 87(2-3): 231-6.
14. Bhatnagar CS, Bhardawaj B, Sharma DK, Meena SK. Incidence of Haemoprotozoan diseases in Cattle in Southern Rajasthan, India. Int. J. Curr. Microbiol. App. Sci., 2015; 4(3): 509-514.
15. Abo El-Kheir EA. Epidemiological aspects on the distribution of haemotropic parasites among cattle in Upper Egypt confirmed by the identification of encountered organisms. M.V. Sci. Thesis. Fac. Vet. Med. Cairo Univ, 1989.
16. Ayub AEM. Studies on some blood parasites in farm animals in Gharbia Governorate. Ph. D. Thesis, Fac. Vet. Med. Kafr El- Sheikh University, 2005.
17. Abbas AA, Abd-Elbaky SM, Ahmed HN, Abdel-Mohsen A, Awaad SE. Seasonal abundance of hard ticks Ixodidae in two localities (Giza and Esmaieliya Governorates) of Egypt. Sci. J. Fac. Sci. Minuf. Univ., 2006; XX: 65 – 87.
18. Loftis AD, Reeves WK, Szumlas DE, Abbassy MM, Helmy IM, Moriarity R, Dasch GA. Rickettsial agents in Egyptian ticks collected from domestic animals. Exp. Appl. Acarol, 2006; 40: 67–81.
19. Fadly RS. Prevalence of blood parasites of some farm animals at Behera Province. Assiut Vet. Med. J., 2012; 58(134): 316-322.
20. Mahmoud MS, Kandil OM, Nasr SM, Hendawy SHM, Habeeb SM, Mabrouk DM, Silva MG, Suarez CE. Serological and molecular diagnostic surveys combined with examining hematological profiles suggests increased levels of infection and hematological response of cattle to babesiosis infections compared to native buffaloes in Egypt. Parasites & Vectors, 2015; 8: 319. DOI 10.1186/s13071-015-0928-9.
21. Cao S, Zhang S, Jia L, Xue S, Yu L, Kamyingkird K. Molecular detection of Theileria species in sheep from northern China. J.Vet. Med. Sci., 2013; 75: 1227-30.
22. El-Sawalhy A. Some studies on cattle blood parasites in Kalubia Province M.V. Sc. Thesis. Infectious diseases, Fac. Vet. Med. Zagazig University; 1987.
23. Vahora SP, Patel JV, Patel BB, Patel SB, Umale RH. Seasonal incidence of Haemoprotozoal diseases in crossbred cattle and buffalo in Kaira and Anand districts of Gujarat, India. Vet. World, 2012; 5(4): 223-225.
24. Chafick ML. Serological tests for the field diagnosis of Babesia infection in calves. M. V. Sc. Thesis, Fac. Vet. Med. Cairo University, 1987.