

Molecular surveillance and phylogenetic analysis of *Theileria annulata* in bovine at Baghdad city/ Iraq

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Summary

This study is conducted to investigate *Theileria* spp. by traditional and molecular methods. A total of 150 blood and 50 lymph samples were collected from local breed symptomatically and asymptotically cattle of both sexes with age ranging from less than 6 months to more than 1 year during the four seasons of 2018, in different parts at Baghdad city / Iraq. Microscopic examination of Giemsa stained blood smears revealed 39.33 % (59/150) rate of infection with bovine theileriosis and 34 % (17/50) positive lymph smears. Statistically no significant difference recorded between female and male: 42.04 % (37/88) and 35.48 % (22/62) respectively. Higher rate of infection 57.97 % (40/69) were recorded in more than 1 year age and 0 % in less than 6 months. 48.93 % (23/47) rate of bovine theileriosis was recorded during summer and 39.53 % (17/43) ; 37.5 % (15/40) rates were recorded during spring and autumn respectively, while the lower rate recorded in winter 20 % (4/20). DNA extraction and polymerase chain reaction (conventional PCR) were done on all cattle blood samples the result recorded that 22 out of 25 samples were positive for *Theileria spp* and *Theileria annulata* with percentage of 88 %. Also DNA sequencing analysis and genetic relationship were conducted by phylogenetic analysis.

Key words: PCR, Theileria, Molecular.

Introduction

Tick borne diseases are related to livestock production in many developing countries including Iraq, and are responsible for high morbidity and mortality which results in decrease production of milk and meat (1). Theileriosis is parasitic tick borne diseases caused by parasite belong to genus *Theileria*, which infect cattle in tropical and subtropical areas (2 and 3). *Theileria* spp. is intracellular parasite cause active latent bovine theileriosis, primary diagnosis of disease based on microscopy as a traditional method, and conventional PCR in case of lower parasitemia and absence of clear symptoms of disease (4).

Bovine theileriosis is caused by most pathogenic *Theileria annulata*, is prevalent and economically disease of cattle and water buffaloes, known as Mediterranean coast fever (5 and 6). Tropical theileriosis or *Theileria annulata* infection is characterized by high fever, weakness, loss of appetite, weight loss, enlarged lymph nodes, anemia, conjunctival petechial paleness, diarrhea in advance stages and recumbency (7 and 8).

Diagnosis of theileriosis is based on clinical signs and is confirmed by examination of

Giemsa stained blood smears for detection of piroplasms in erythrocytes and schizonts in lymphocytes (9). Molecular diagnosis is used as rapid identification of Theilerial infection in animals give a negative result in serological tests and still infected with ticks (10 and 11).

Al-Khaledi (12) recorded 32.93 % rate bovine theileriosis and recorded mixed infection with *Theileria* and *Babesia* 16.78 % in Al-Qadisiya / Iraq, while (13) recorded 13.23 % rate of infection with theileriosis in cattle by microscopic examination and recorded 33 positive cases of 100 blood samples by PCR analysis in Al-Muthanna province, Iraq.

This study was conducted for detection and investigation *Theileria* spp. in local breed Iraqi cattle, in different areas of Baghdad city using microscopic examination of stained blood smears, molecular technique, DNA sequence and phylogenetic tree analysis.

Materials and Methods

A total of 150 blood samples were collected from Jugular vein of each local breed both sexes Iraqi cattle suffered from weakness, anemia, high fever and high infestation of ticks and other asymptomatic with age ranging from

less than 6 months to more than one year along four seasons of 2018 also 50 lymph samples were aspirated from pre-scapular lymph nodes of same animals blood samples were collected in anticoagulant tubes (EDTA) for microscopic and molecular detection of Theileria parasite after transferred in cooling box to parasitology laboratory at Veterinary Medicine College, University of Baghdad/ Iraq.

Thin blood and lymph smears were prepared and stained by Giemsa stain (10 %) and examined under oil immersion (X 100) according to (14).

Genomic DNA from cattle blood samples were extracted by using (Quantipher Genomic DNA Extraction spin kit-Turkey) and done according to company recommendations. The blood Genomic DNA checked by using Nanodrop spectro photometer that measured DNA concentration and purity at (260/280 nm) absorbance.

Conventional PCR by specific primers was used for detection *Theileria spp.* and *Theileria annulata* (15) provided by IDT Canada. (Table, 1). The preparation of PCR master mix was : 10 µl PCR premix , 3 µl from forward and reverse primer , 5 µl of DNA template and

2 µl of PCR water for final volume of 20 µl . Conventional PCR thermocycler system was done as steps (Table, 2). The PCR products were analyzed by agaros gel electrophoresis and visualized using ultraviolet Trans illuminator.

DNA Sequencing was performed to confirm the detection of local *Theileria spp.* and *Theileria annulata* isolates in cattle, and for phylogenetic relationship analysis of 18 ribosomal RNA and cytochrome b genes respectively with NCBI–Gene Bank Global. The PCR sequence result were edited and analyzed by (Mega x) program and using nucleotide collection (nt/nr) database , also to specify the parasite blasted sequences (Neighbor-joining method) were used for aligned sequences. phylogenetic analysis were carried out to find the relationship of sequences determined in the study obtained along with relevant sequences deposited in Genbank DNA Sequencing carried out by (Suol–Korea).

Data were analyzed by using SAS program (2014) the proportions were compared using Chi-square test, P ≤0.05 is considered as significant.

Table, 1: Primers and their sequence that used in study.

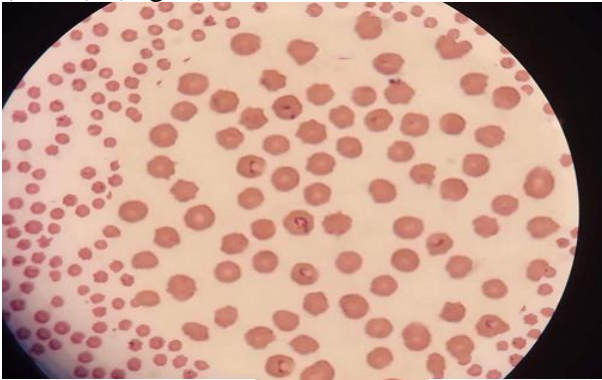
Primers	Sequence (5` – 3`)	Product Size (bp)
<i>Theileria spp.</i> 18s ribosomal RNA Gene	F: 5` – AGTTTCTGACCTATCAG – 3` R: 5` – TTGCCTTAAACTTCCTTG – 3`	1100
<i>Theileria annulata</i> Cytochrome b	F: 5` – ACTTTGGCCGTAATGTTAAAC – 3` R: 5` – CTCTGGACCAACTGTTTGG – 3`	312

Table, 2: PCR Thermocycler system (Amplification program).

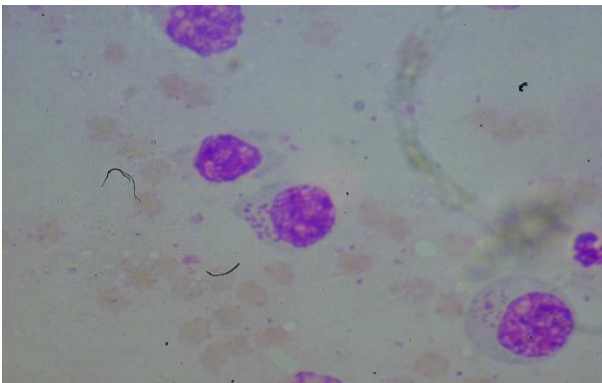
Gene	Initial Denaturation	Cycle Conditions			Final Extention	Cycle No.
		Denaturation	Annealing	Extention		
<i>Theileria spp.</i>	94 °C/3min	94 °C/30 sec	52 °C/30 sec	72 °C/30 sec	72 °C/7 min	35
<i>Theileria annulata</i>	94 °C/3 min	94 °C/30 sec	58 °C/30 sec	72 °C/30 sec	72 °C/5 min	35

Results and Discussion

The overall rate of infection with bovine theileriosis after examining 150 blood smears was 39.33 % (59/ 150). The piroplasm forms were detected in the positive animals as in (Fig. 1), also 17 animals showed positive lymph smears for schizont stage with rate of 34 % (17/50), (Fig. 2).



Figure, 1: Erythrocytic stage, Piroplasm of Theileria spp. In cattle blood smear (x 100).



Figure, 2: Lymphocytic stage, Schizont of Theileria spp in cattle lymph smear (x 100).

The total rate of infection with bovine theileriosis in different parts of Baghdad city / Iraq was 39.33 % by microscopic examination of 150 Giemsa stained blood smears. This result was agreed with that recorded by (12) in AlQadisiya province / Iraq 32.93 % and higher than 12.23 % that recorded by (13) in Al-Muthana province / Iraq also the result was approximately near the rates 33.3 % and 33.5 % which recorded by (16) in Sudan. The differences were attributed to the numbers of animals examined and differences in climate which favorite for tick infestation, also the prevalence of infection influenced by breed of cattle that showed differences in tick resistance and innate susceptibility to infection(17).

Female and male recorded 42.04 % (37/88) and 35.48 % (22/62) infection rates respectively without significant difference P= 0.50 (Table ,3).

Table, 3: Infection rate of bovine theileriosis by microscopic examination according to sex.

Sex	No .of animals examined	No. of positive	%
Female	88	37	42.04
Male	62	22	35.48
Total	150	59	39.33

X2 : chi square : 0.45P=0.50.

Infection rates of bovine theileriosis in this study didn't affected by sex of animals, and this coincided with studies of (12, 18 and 19). Higher rate of infection with bovine theileriosis was recorded in animals with more than 1 year age group 57.97 % (40/69), while 0 % (0/30) recorded in less than 6 months age group with significant differences P>0.0001 (Table ,4).

Table, 4: Infection rate of bovine theileriosis by microscopic examination according to age groups.

Age groups Month/Year	No .of animals examined	No. of positive	%
< 6 Months	30	0	0
6 Months – 1 Year	51	19	37.25
> 1 Year	69	40	57.97
Total	150	59	39.33

X2 : chi square : 35.86P>0.0001

Our results recorded higher rate of infection in 1 year age group in a coordinate with study of (20) who showed that the infection with Theileria parasite increase with the age. This is might be due to subsequent exposures to infested ticks that represent the important

source of infection (21). Summer showed highest rate of infection with Theileria spp 48.93 % (23/47), spring and autumn recorded 39.53 % (17/43) and 37.5 % (15/40) respectively, the lower rate recorded in winter 20 % (4/20) (Table, 5).

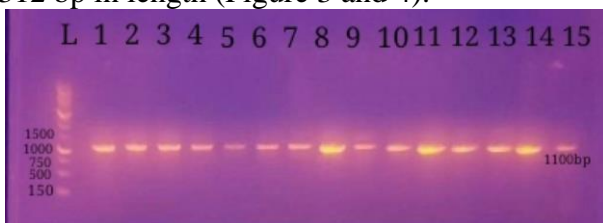
Table, 5: Infection rate of bovine theileriosis by microscopic examination according to seasons of study.

Season	No .of animals examined	No. of positive	%
Spring	43	17	39.53
Summer	47	23	48.93
Autumn	40	15	37.5
Winter	20	4	20
Total	150	59	39.33

X2 : chi square : 5.006P=0. 17.

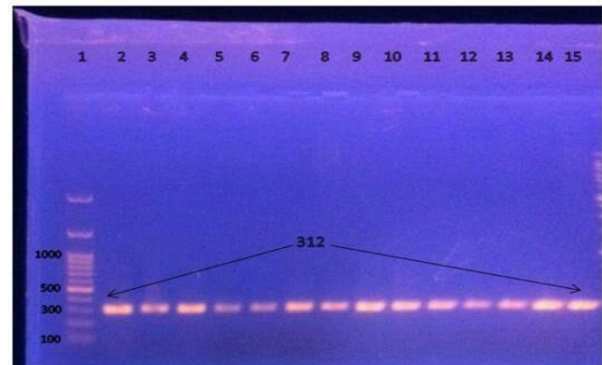
According to seasons, bovine theileriosis was more prevalent in summer 48.93 %, spring and autumn recorded nearest rates 39.53 % and 37.5 respectively, these result agreed with (12) in Al-Qadisiya province/Iraq who recorded 74.77 % and 71.77 % during summer and spring respectively prevalence increase with warm moist climate that is favorable environment for Theileria sporozoites develop as a result of (22), tick's activity increase in summer, and as a result the salivary gland of single tick contain large amount of sporozoites which can be fatal (7).

Out of 25 blood samples examined by conventional PCR technique 22 was positive for Theileria SPP., that revealed PCR product of 1100 bp in length , and 22 was positive for Theileria annulata that revealed PCR product of 312 bp in length (Figure 3 and 4).



Figure, 3: Agarose gel (1.5 %) electrophoresis of amplified DNA from Theileria spp. lane M: 1

– 15, DNA Ladder (100-3000 bp) some positive product showed clear band in 1100 bp.



Figure, 4: Agarose gel (1.5 %) electrophoresis of amplified DNA from Theileria annulata. lane M: 1–15, DNA Ladder (1000 bp) some positive product showed clear band in 312 bp.

DNA sequencing result:Twenty samples purified by PCR technique analyzed by sequencing to get nucleotide sets of (18s ribosomal RNA) gene (1100 bp) for Theileria spp. And (cytochrom b) gene (312 bp) for Theileria annulata isolated from cattle in different parts of Baghdad city /Iraq and recorded in gene bank with accession numbers: MK 182862 , MK 182863 , MK 182864 , MK 182865 , MK 182866 ,MK 182867 , MK 182868 , MK 182869 , MK 182870 , MK 182871.

The analysis of phylogenetic tree based on sequences of(1100 bp) 18 s ribosomal RNA gene, and (312 bp)cytochrom b gene for Theileria spp. and Theileria annulata respectively. phylogenetic tree using constructed neighbor joining bootstrab1000 radiation tree. Analysis result showed that homology of nucleotides sequences between local isolate of Iraqi Theileria spp. was nearly closed to India , Spain , Turkey , Tajikistan , China , Iran , and Pakistan isolates (MF 287926 , FJ426369 , MG569892 , KM288519 ,KF 559356 , KF429794 and JQ 743634) respectively with homology sequence identity 99% (Figure,5 and 6).

Theileria species can be differentiate by Molecular tools, this technique have proved to be highly sensitive and specific in detecting blood parasite DNA (23). Piroplasm in carrier animals determine by using diagnostic Molecular species–specific assay in veterinary parasitology (24 and 25). PCR have been used

to detect and differentiate *Theileria* spp. In carrier and clinically infected animals, even with very low amount of parasite DNA .

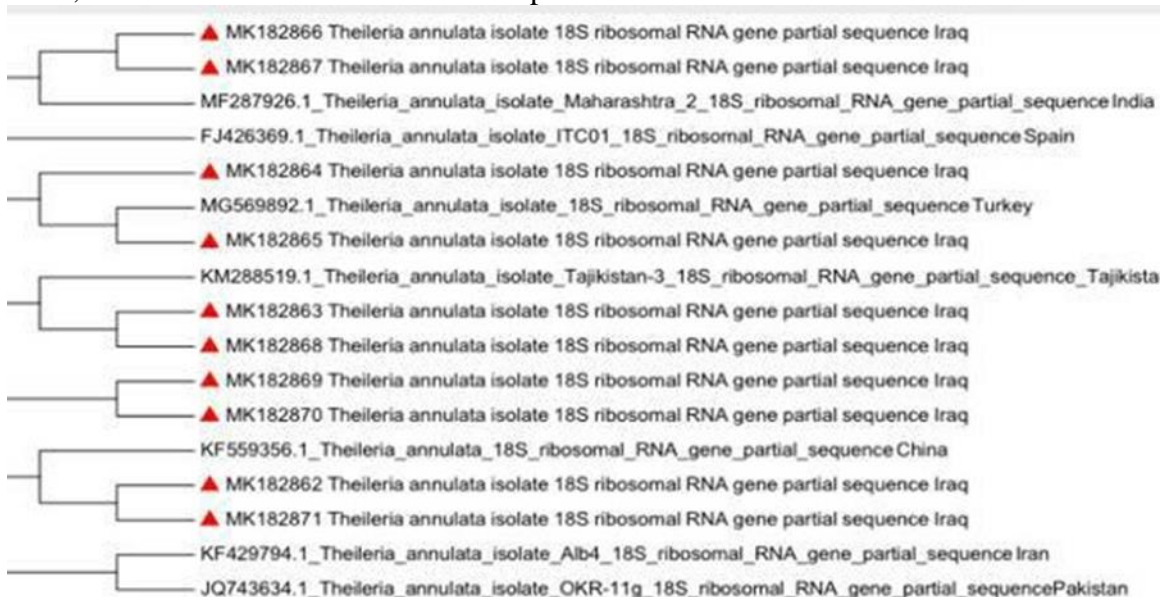
PCR technique showed high rate of infection with *Theileria* spp. and in different parts of Baghdad city, using specific primers: 18s ribosomal RNA gene (1100 bp) and cytochrom b gene (312 bp), that was accurate and species-specific (GenBank , MG 787986.1 and KP 731977.1). The results were higher than that recorded by (26) 68.9 % and (19) 88.23 % in Kurdistan and Basrah province/Iraq respectively and higher than (21) in Algeria 30.16 % and (27)in Egypt 24.05%.

The conventional PCR result using 18s rRNA gene and confirmed our result that detected *Theileria* spp. by examining Giemsa Stained blood smears , also PCR is a successful and sensitive method in detection *Theileria annulata* by using cytochrom b gene . The highest percentage rate recorded by PCR was good indication for highest prevalence of bovine theileriosis in Baghdad .

Our result recorder 88% rate by PCR and 39.33% microscopically, these agreed with many researchers, (28) in Turkey and (29) in India, the differences between microscopic and

molecular methods attributed to several reasons as chronic and carrier cases with low parasitemia and parasite misdiagnosis with staining artifacts(30).

Phylogenetic analysis confirmed low differences between Iraqi strains of *Theileria annulata* and other countries .The genetic variation that recorded may be due to variation on area size of reference sequence and differences in geographical areas where isolates collected. Using different methods like PCR based on gene sequencing of partial or complete genes, Phylogenetic have been used for genetic analysis. When environment of parasite change, genetic diversity plays important role in survival and makes accurate analysis of variation applicable for studies on taxonomy, biology, epidemiology and pathogenesis of parasites. Result of this study agreed with some studies that reported the existence of genetic variation and phylogenetic relationships based on mitochondrial and nuclear gene sequences among populations of parasite that analyze genetic variations of *Theileria* protozoa in the world .



Figure, 5: Analysis of phylogenetic tree based on the partial sequence small subunit of 18s rRNA of *Theileria* spp. , local and global sequence using neighbor joining bootstrab 1000 radiation tree figure evolutionary relationships.



Figure, 6: Analysis of phylogenetic tree based on the partial sequence small subunit of cytochrom b of *Theileria annulata*, local and global sequences using neighbor joining bootstrab 1000 radiation tree figure evolutionary relationships.

Results of study explained that *Theileria* spp. distributed in local breed cattle in different areas at Baghdad city/Iraq according to microscopic examination of Giemsa stained blood smears and PCR technique. The molecular and phylogenetic analysis are very sensitive and accurate in determining parasite species. The scientific diagnostic molecular methods easily task production of vaccines and proper use of different treatments to prevent spreading of disease.

References

1. George N, Bhandari V, Reddy DP. and Sharma P. (2015). Molecular and Phylogenetic analysis revealed new genotype of *Theileria annulata* parasites from India. *Parasitology Vectors* 8, 468. DOI: 10.1186/s13071-015-1075-z
2. Nazifi S, Razavi SM, Esmailnejad Z. and Gheisari H. (2009). Study on acute phase proteins (haptoglobin, serum amyloid A, fibrinogen, and ceruloplasmin) changes and their diagnostic values in bovine tropical theileriosis. *Parasitology Research* 105, 41–46. DOI: 10.1007/s00436-009-1360-x. Epub 2009 Feb 24
3. Demessie Y. and Derso, S. (2015). Tick Borne Hemoparasitic Diseases of Ruminants: A Review of Advance Biological Research 9(4):210-224. DOI: 10.5829/idosi.abr.2015.9.4.9516
4. Debabrata D, Vasundhra B, Sreenivasamurthy GS, Shweta M, Sonti R, Vandna B, Jaspreet SA, Satparkash S. and Paresh S. (2018). A Real-Time PCR based assay for determining parasite to host ratio and parasitaemia in the clinical samples of Bovine Theileriosis. *Scientific Reports* 8,15441. <https://doi.org/10.1038/s41598-018-33721-3>
5. Slodki J, Jasik KP, Kepa M, Idzik D. and Wojtyczka R. (2011). Tick-transmitted disease caused by apicomplexa. *Acta Protozoologica* 50: 155–161.
6. Erdemir A, Aktas M. and Dumanli N. (2012). Isolation, cloning and sequence analysis of the lactate dehydrogenase gene from *Theileria annulata* may lead to design of new anti Theilerial drugs. *Veterinary Medicine* 57(10) and 559-567. DOI: 10.17221/6368-VETMED
7. Radostits OM, Gay CC, Hinchcliff KW. and Constable PD. (2007). *Veterinary Medicine: A textbook of the diseases of cattle, horses, sheep, pigs and goats*, 10th Ed. Elsevier, Philadelphia, USA,1522-1532.
8. Muhanguzi D, Picozzi K, Hatendorf J, Thrusfield M, Welburn SC, Kabasa,

- JD, Waiswa C. (2014). Prevalence and spatial distribution of *Theileria parva* in cattle under crop-livestock farming systems in Tororo District, Eastern Uganda. *Parasites and Vectors* 9(91), 1–8. DOI:10.1186/1756-3305-7-91
9. Nourollahi-Fard SR, Khalili M. and Ghalekhani N. (2013). Detection of *Theileria annulata* in blood samples of native cattle by PCR and smear method in Southeast of Iran. *Journal of Parasitic Diseases* 39(2) :249–252. DOI: 10.1007/s12639-013-0333-2
 10. Zaeemi M, Haddadzadeh H, Khazraini P, Kazemi B. and Bandehpour M. (2011). Identification of different *Theileria* species (*Theileria lestoquardi*, *Theileria ovis*, and *Theileria annulata*) in naturally infected sheep using nested PCR–RFLP. *Parasitology Research* 108:837–843. DOI: 10.1007/s00436-010-2119-0
 11. Ghaemi P, Hoghooghi-Rad N, Shayan P. and Eckert B. (2012). Detection of *Theileria orientalis* in Iran by semi-nested PCR. *Parasitology Researcher* 110:527-531. DOI: 10.1007/s00436-011-2517-y
 12. Alkhaledi MJA. (2008). Epidemiological study of theileriosis, babesiosis and anaplasmosis in cattle of Al Qadisiya province. MSc thesis, College of Veterinary Medicine, University of Baghdad, Iraq.
 13. Tallaf AK. (2017). Detection of Theileriosis in cattle by using polymerase chain reaction in AL-Muthanna Province. MSc Thesis in Veterinary Medicine, Baghdad University, Iraq.
 14. Chaudhri SS. and Gupta SK. (2003). *Manual of General Veterinary Parasitology*. 1st Ed. Department of Veterinary Parasitology College of Veterinary Science. Haryana Agricultural University, India, pp: 46-47.
 15. El-Naga TRA. and Barghash SM. (2016). Blood parasites in Camels (*Camelus dromedarius*) in Northern West Coast of Egypt. *J. of Bacteriology and parasitology* 7(1): 1-7. DOI: 10.1016/j.parepi.2016.07.002
 16. Salih DA, Hussein AM, Seitzer U. and Ahmed JS. (2007). Epidemiological studies on tick-borne diseases of cattle in central equatorial state, southern Sudan. *Parasitology Research* 101(4):1035–1044. DOI:10.1007/s00436-007-0583-y
 17. Muhammad G, Naureen, A, Firyal S. and Saqib M. (2008). Tick control strategies in dairy production medicine. *Pakistan Vet. J.*, 28(1): 43-46.
 18. Al-Robayi HM. (1999). Epidemiology of *Theileria annulata* infection in Al Ashaiki farm. PhD Thesis, College of Veterinary Medicine, University of Baghdad.
 19. Al-Emarah GY, Khudor MH. and Daham HR. (2012). Clinical, haematological and biochemical study to cattle naturally infected with *Theileria annulata* north of Basrah province. *AL-Qadisiya. J. of Vet. Med. ISci.* 11(1): 54-62.
 20. Darghouth MA, Mhadhbi M, Naouach A, Boumiza A, Chaabani MF. and Ben-Abderazzak S. (2010). In vivo evidence for the resistance of *Theileria annulata* to buparvaquone. *Veterinary Parasitology* 169: 241–247. <https://doi.org/10.1016/j.vetpar.2010.01.013>
 21. Ziam H, Kelanamer R, Aissi M, Ababou A, Berkvens D. and Geysen D. (2015). Prevalence of bovine theileriosis in North central region of Algeria by real-time polymerase chain reaction with a note on its distribution. *Tropical Animal Health and Production* 47(5):787-796. DOI: 10.1007/s11250-015-0772-0
 22. Haque M, Singh NK. and Rath SS. (2010). Prevalence of *Theileria annulata* infection in *Hyalomma anatolicum anatolicum* in Punjab state, India. *Journal of Parasitic Diseases* 34(1): 48-51. DOI: 10.1007/s12639-010-0004-5
 23. Tarimo MA. (2013). Studies on the prevalence of East Coast Fever among

- cattle in Kilosa district. PhD thesis, Agriculture Sokoine University.
24. Yusufmia SB, Collins NE, Nkuna R, Troskie M, Van den Bossche P. and Penzhorna BL. (2010). Occurrence of *Theileria parva* and other haemoprotozoa in cattle at the edge of Hluhluwe-iMfolozi Park, KwaZulu-Natal, south Africa. *J. of the South African Veterinary Assoc.* 81(1): 45-49.
 25. Gul N, Ayaz S, Gul I, Adnan M, Shams S. and Akbar N. (2015). Tropical Theileriosis and East Coast Fever in Cattle: Present, Past and Future Perspective. *International J. of Current Microbiol. and Applied Sci.* 4(8): 1000-1018. DOI: 10.9790/2380-1105020709
 26. Al-Saeed ATM, Omer LT, Abdo J, Habibi G, Salih D A, Seitzer U. and Ahmed J. (2010). Epidemiological studies on tropical theileriosis (*Theileria annulata* infection of cattle) in Kurdistan region, Iraq *Parasitol. Res.* 106(2):403-404. <https://doi.org/10.1007/s00436-009-1675-7>
 27. Nayel M, El-Dakhly KM, Aboulaila M, Elsify A, Hassan H, Ibrahim E. and Yanai T. (2012). The use of different diagnostic tools for *Babesia* and *Theileria* parasites in cattle in Menofia, Egypt. *Parasitol. Res.* 111(3): 1019-1024. DOI: 10.1007/s00436-012-2926-6. Epub 2012 Apr 29
 28. Aktas M, Altay K. and Dumanli N. (2006). A molecular survey of bovine *Theileria* parasites among apparently cattle and with a note on the distribution of ticks in eastern Turkey. *J. of Vet. Parasitology* 138:179-185. <https://doi.org/10.1016/j.vetpar.2006.01.052>
 29. Kohli S, Atheya UK. and Thapliyal A. (2014). Prevalence of theileriosis in cross-bred cattle: its detection through blood smear examination and polymerase chain reaction in Dehradun district, Uttarakhand, India. *Veterinary World* 7(3): 168–171. DOI: 10.14202/vetworld.2014.168-171
 30. Charaya G, Rakha NK, Maan S, Kumar A, Kumar T. and Jhambh, R. (2016). Comparative evaluation of polymerase chain reaction assay with microscopy for detection of asymptomatic carrier state of theileriosis in a herd of crossbred cattle. *Vet. World* 9(9): 1039-1040. doi: 10.14202/vetworld.2016.1039-1042

التوصيف الجيني وتحليل الشجرة الوراثية لطفيلي *Theileria annulata* في ابقار في مناطق مختلفة من مدينة بغداد / العراق

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الخلاصة

اجريت الدراسة للتحري عن طفيلي *Theileria spp*. باستخدام الطرق التقليدية والجينية. جمعت 150 عينة دم و 50 عينة لمف من ابقار لعروق محلية ومن كلا الجنسين وذات فئات عمرية تراوحت اقل من 6 اشهر الى اكثر من سنة خلال الفصول الاربعة للعام 2018 ومن مناطق مختلفة في العراق. اظهر الفحص المجهرى للمسحات الدموية المصبوغة بالكمز نسبة اصابة كلية بـثايليريا الابقار (150/59) 39.33%، وسجلت المسحات اللمفية الموجبة نسبة (50/17) 34%. احصائياً لم تسجل فروق معنوية بين الاناث (88/36) 40.90% والذكور (62/22) 35.48%. اعلى نسبة اصابة (40.69) 57.97%. سجلت في اعمار اكثر من سنة و نسبة 0% كانت في اقل من 6 اشهر. وكانت نسبة الاصابة بـثايليريا الابقار اعلى في فصل الصيف (47/23) 48.93% وكانت النسبة (43/17) 39.53% في الربيع و (40/15) 37.5% في الخريف و اقل نسبة كانت في فصل الشتاء (20/4) 20%. اظهرت نتائج استخلاص الحمض النووي DNA وفحص تفاعل سلسلة البلمرة ان هناك 22 عينة من اصل 25 عينة موجبة لكل من جنس طفيلي *Theileria* ونوع *Theileria annulata* وبنسبة 88%. كما اجري التسلسل والعلاقة الجينية للعينات عن طريق تحليل الشجرة الوراثية. الكلمات المفتاحية: تفاعل سلسلة البلمرة، التثرييا، التوصيف الجيني.