Kufa journal for Veterinary Medical Sciences Vol.(9) No.(2)

Kufa journal for Veterinary Medical Sciences

Kj.vs@uokufa .edu.iq

www.uokufa .edu.iq\journals\index.php\kjvs

Molecular study of *Babesia spp* and *Theileria spp* in camels of Al-Diwaniyah province in Iraq

Zainab Hussain Chyad Al-Naily Ghaidaa Abbas Jasim

Coll. of Vet. Med. / Univ. of Al-Qadissiay

email: zainhussein85@yahoo.com ghydaaaa@yahoo.com

Received date:9June 2018 **Accepted:**(435) 24 Sept 2018 **page** (62-70) **Pubished:**31 Dec 2018

Abstract

Because of the economic importance of the camels and the scarcity of studies related to it in Iraq, our study aimed to investigate *Babesia spp* and *Theileria spp* by molecular methods. The current study was conducted during the period from September 2017 to March 2018 and collected 200 random blood samples from camels included (125 females and 75 males and two groups of ages)150(>1 years) and 50(<1year) some of them appear on it clinical signs and some did not show any symptoms from the slaughterhouse of Al- Diwaniya province. This study was designed to diagnose these parasites firstly by microscopic examination by Geimesa stain method .The results each of (106/200)(53%) *Babesia spp* and (93/200)(46.5%) *Theileria spp*. The prevalence of Babesiosis in the female (76/125)(60.8%) more than in the males (30/75)(40%) and in different ages. Theileriosis also recorded the prevalence in female (69/125)(55.2%) and in males (24/75)(32%) and in different ages. The highest incidence in months (September, October and March) (88.3%, 96.2% and 75%), respectively, according to the study time. recorded mix infection 19(23.75%) between *Babesia* &*Theileria* then diagnosis 90 blood samples positive microscopically using molecular techniques, which included (conventional PCR monoplex and multiplex) 16 (17.77%), 9(10%), 8(8.9%) and 11(12.22) *Theileria spp* , *Theileria annulata*, *Babesia bovis* and *Babesia bigemina* respectively .

Key words : PCR, Babesia , Theileria ,camels

دراسة جزيئيه لطفيليات البابيزيا والثليريا في الجمال في محافظة الديوانية في العراق

زينب حسين جياد النائلي غيداء عباس جاسم

كلية الطب البيطري, جامعة القادسية فرع الطفيليات ,

الخلاصة

نظرا للأهمية الاقتصادية للابل وبسبب قلة الدراسات التي تتعلق بها في العراق جائت هذه الدراسة للتحري عن البابيزيا و الثليريا بالطرق الجزيئية . أجريت هذه الدراسة خلال الفترة الممتدة من شهر أيلول 2017 ولغاية شهر اذار 2018 تم جمع 200 عينة دم عشوائية من الابل (125) اناث و(75) ذكور وبمجموعتين من الاعمار(150)عينه اكبر من سنه و(50) عينه اصغر من سنه ومنها ظهرت عليها علامات سريرية ومنها لم تظهر عليها اي اعراض من مجزرة محافظة الديوانية .صممت هذه الدراسة لتشخيص هذه الطفيليات اولا بالفحص المجهري باستخدام صبغة كمزة(Geimesa stain) وكانت النتائج (200/106)(53%) بابيزيا و (200/93)(46.5%) ثيليريا. من مجموع 200 عينه. سجلت اعلى نسبة اصابة بداء الكمثريات في الاناث اكثر من الذكور (76/ 125)(60.8%), (30/ 75)(40%) وبمختلف الاعمار. الثيليريا ايضا سجلت اعلى نسبة اصابة في الاناث اكثر من الذكور(125/69) (55.2%) و (24/ 75)(32%) وبمختلف الاعمار . سجلت اعلى نسبة اصابه في الاشهر (ايلول ,تشرين الاول واذار) (88.3% , 96.2% و75%) على التوالي حسب وقت الدراسة . وسجلت اصابة مشتركة ما بين البابيزيا والثليريا وبنسبة 19(23.75%) .ثم تشخيص 90 عينة دم

موجبة مجهريا باستخدام التقنيات الجزيئية والتي تضمنت تفاعل السلسلة البلمرة الاعتيادي) وكانت النتائج كالاتي, *Theileria spp , Theileria annulata Babesia bovis, Babesia bigemina* وبالنسب التالية 16(17.77%), 9 (10%) و 8(8.9%),11(12.22%) على التوالي.

الكلمات المفتاحية :اختبار تفاعل سلسلة البلمرة الاعتيادي ,بابيزيا ,ثليريا , الجمال

Introduction

camel is consider to be one of best farm animals can tolerated the harsh conditions in this arid parts of the world because of the unique adaptive the physiological characteristics(1). It has an important role in economic and breeding, when requires good managements and the control programs of the diseases (2).Camels are multi-use animal used for transport and product of milks, meats and wools since ancient times (3). Many parasitic diseases can infected the camels and this affect their healthy and cause signs as anemia , wasting, fever and deaths (4).Camel medicine has very long history in world and Iraq (5). These diseases are transmission from camel to camel by vectors as hard ticks which transmitted the blood parasites(6,7,8). Theileriaosis was more importance disease of animals induce vary clinic appearance as fever, superficial lymph node enlargement, lacrimation, a sudden loss of condition and loss appetite (9,10). Tropical Theileriosis caused by *Theileria spp* has a broad distributed extended from North Africa - China (11,12). Piroplasmosis belonged to the genus *Babesia* are suspect of infecting camels (13). The first recorded infection in camels with *Babesia spp* specially *B. caballi* in Egypt(14) and in Iraq by (15). The typical appearance of Babesiosis are fever, anemia, icterus , gastro intestinal stasis and heamoglobin urea (3,13). The most common methods for diagnosis of blood parasites are checking the blood electron microscopy and then DNA extraction for is confirm diagnosis by the use of technologist (PCR) with analysis sequence(13). There are no control programs to prevent blood parasites on a large scale(12). Molecular tools and material increase have become completed part of studying epidemiology and diagnosis of blood parasites (16) . This study was conducted to investigate these parasites of the local Iraqi camels in Al-Diwaniyah governorate to detection some blood parasites based on microscopic examination , Molecular diagnosis by using a polymerase chain reaction technique (PCR) and Phylogenetic tree to domestic blood parasites in Iraqi camels .

Materials and Methods

Blood samples collection

Total of 200 blood samples were collected from jugular vein of each camel and kept tubes with (EDTA) randomly from camels in the slaughterhouse in Al-Diwaniyah province during the period from September – 2017 to the end of march – 2018 and data included animal's age (>1 year and <1 year) and both sex .

Parasitological examination:

A drop of blood was taken and put on the glass microscopic slide and spread by another slide then left in air to dray fixed by absolute methanol for (5-10)minute then stained with Giemsa stain for 30 mints then washed by tab water and left to dry after drying they examined under the oil immersion lens of light microscope according to (3).

Genomic DNA Extraction

Genomic DNA from camel blood samples were extracted by using Column-pure blood Genomic DNA Mini Kit (Abm) Canada .

Molecular methods

Used conventional PCR by specific primers for detection *Babesia spp* and *Theileria spp* and PCR thermo cycler system as following table(1).

Table (1): PCR amplification program

Gene	Initial Denaturation	Cycling conditions			Final extension	Hold	Cycle No.
		Denaturation	Annealing	Extension			
Babesia bovis	**94°C/280sec**	**94°C/120sec**	**59/60sec**	**72°C/60sec**	**72°C/60sec**	**4°c**	**40**
Babesia bigemina	**94°C/280sec**	**94°C/120sec**	**58/60sec**	**72°C/60sec**	**72°C/60sec**	**4°c**	**40**
Theileria annulata	**94°C/280sec**	**94°C/120sec**	**58/60sec**	**72°C/60sec**	**72°C/60sec**	**4°c**	**40**
Theileria sp.	**94°C/280sec**	**94°C/120sec**	**57/60sec**	**72°C/60sec**	**72°C/60sec**	**4°c**	**30**

Primers

Specific primers provided by IDT(Canada) as(table -2).

Table-2 Primers used in this study with their Sequence and PCR size

Primer	Sequence (5'-3')		product Size (bp)	source
Babesia bovis **glutamine-dependent carbamoyl phosphate synthase (CPSII)**	**F**	**TTTGGTATTTGTCTTGGTCAT**	**446-453bp**	**17**
	R	**ACCACTGTAGTCAAACTCACC**		
Babesia bigemina **18ribosomal RNA gene**	**F**	**TAGTTGTATTTCAGCCTCGCG**	**689bp**	**18**
	R	**AACATCCAAGCAGCTAHTTAG**		
Theileria annulata **cytochrome b**	**F**	**ACTTTGGCCGTAATGTTAAAC**	**312bp**	**17**
	R	**CTCTGGACCAACTGTTTGG**		
Theileria spp. **18ribosomal RNA gene**	**F**	**AGTTTCTGACCTATCAG**	**1100bp**	**17**
	R	**TTGCCTTAAACTTCCTTG**		

DNA sequencing Analysis

DNA sequencing technique was carried out for Phylogenetic relationship analysis study of genes in camels with NCBI-Gen Bank Global. PCR product carried out the DNA sequencing by (suol-South Korea)

Statistical analysis

Statistical analysis was achieved by using Chi Square tests (X^2)at $p \leq 0.05$ used to analyze differences in these parasites rate among samples, sex and ages (19).

Result

Result of Microscopic Examination.

Out of 200 camels blood samples exam microscopically the result *Babesia spp* (106/200)(53%) and *Theileria spp* (93/200)(46.5%) according to sex and age as in table (3,4,5,6) and figure (1,2).

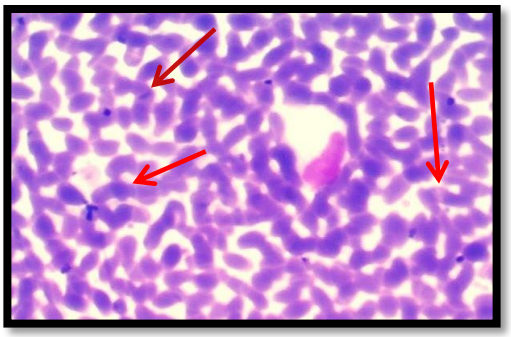


Fig -1 *Babesia spp*. Show inside a camel red blood cells (thick smear) 100x

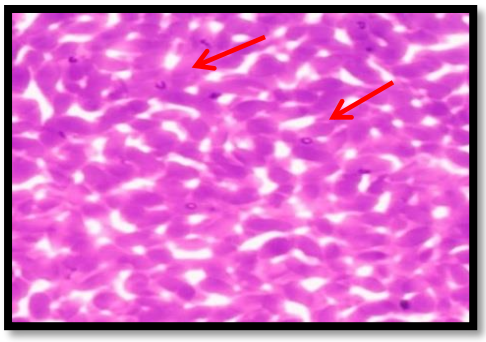


Fig -2 *Theileria spp*. Show inside a camel red blood cells (thick smear) 100x

Table -3 The prevalence of *Babesia spp* infection in camel according to the microscopic examination and sex

Sex	No .of samples	*Babesia spp* infection	
		+	%
Males	75	30	40 ^{B}
Females	125	76	60.8 ^{A}
Total	200	106	53

Different letter = significant difference at $p < 0.05$

Table -4 The prevalence of *Babesia spp* infection in camel according to the microscopic examination and age

age	No. .of samples	*Babesia spp* infection	
		+	%
< 1	50	20	40^{B}
> 1	150	86	57.33^{A}
Total	200	106	53

Different letter = significant difference at $p < 0.05$

Table -5 The prevalence of *Theileria spp.* infection in camel according to the microscopic examination and sex

Sex	No .of samples	*Theileria spp* infection	
		+	%
Males	**75**	**24**	**32^{B}**
Females	**125**	**69**	**55.2^{A}**
Total	**200**	**93**	**46.5**

Different letter = significant difference at $p < 0.05$

Table - 6The prevalence of *Theileria spp* infection in camel according to the microscopic examination and age

Animals age	No.of samples	*Theileria spp* infection	
		+	%
< 1	50	18	36[A]
< 1	150	75	50[A]
Total	200	93	46.5

Similar letter = no significant difference at $p < 0.05$

Result of PCR technique:

Out of 90 positive blood samples microscopically were examined by PCR technique. The results were 8 (8.9%) *Babesia bovis* , 11(12.22%) *Babesia bigemina* ,16(17.77%) *Theileria spp* and 9 (10%) *Theileria annulata* .There are significant difference at $p < 0.05$ and recorded our strains in Gene bank with accession numbers (MH508091, MH508092, MH508093, MH482935, MH482934, MH482936, MH508088, MH508089 and MH508090)

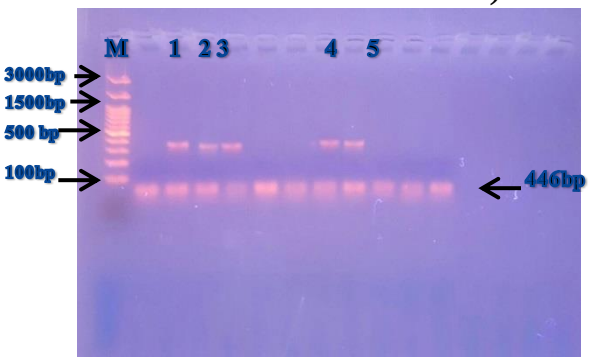
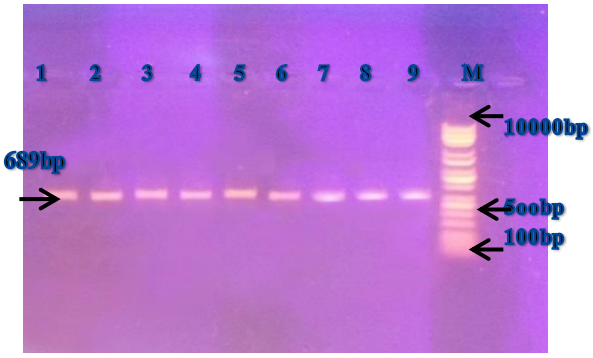


Figure (3): Agarose gel electrophoresis image that show PCR product that amplify fragment of small subunit ribosomal RNA gene in *Babesia bovis* from camel blood samples. Where M: Marker(100bp-1000bp) and line (1-5)some of positive samples for *Babesia bovis* at 446bp product size.



Figure(4) : Agarose gel electrophoresis image that show PCR product that amplify fragment of small subunit ribosomal RNA gene in *Babesia bigemina* from camel blood samples. Where M: Marker (100bp-3000bp) and line (1-9) some of positive samples for *Babesia bigemina* at 689bp product size.

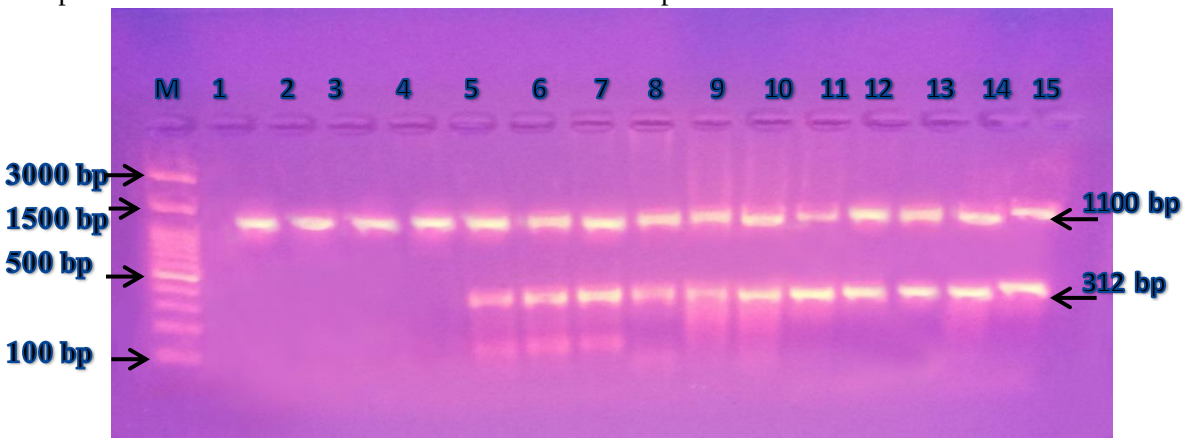


Figure (5): Agarose gel electrophoresis image that show PCR product that amplify fragment of small subunit ribosomal RNA gene in *Theileria spp* and *Theileria annulata* from camel blood samples. Where M: Marker (100bp-3000bp) and line (1-15)some of positive samples for *Theileria spp* at1100bp and *Theileria annulata* at 312bp product size.

Discussion

The study results showed that the local camels were infected with *Babesia spp* and *Theileria spp* and this fact confirm that the disease is widespread and affects all animals species including camels(13). The microscopic examination of the blood smear showed that the *Babesia spp* and *Theileria spp* appear in several shapes inside RBCs which find are agreement with (3,14) . The prevalence of *Babesia spp* in camels using microscopic examination according to sex of animals revealed that the highest rate of infection in females (76/125) (60.8 %) while the lowest rate was in males (30/75) (40%) with there was significant difference at $p < 0.05$. The study results are agree with (17,20,21,22). But disagree with (23)who recorded (31/90)(34.44%)in males camels in AL-Najaf of Iraq. The differences could be due to the several effects such as the time of study, number of samples, location, stress during gestation and milk production performance them more susceptible to *Babesia spp* . While according to the age of camels the highest rate of infection in camels more (>1)year (86/150)(57.33%) while the lowest rate was camels less than (<1) year (20/50)(40%) with there was significant difference at $p < 0.05$. The study results are similar with (23,24). But disagree with result of (26) recorded (28%) in domesticated animals(>1) in north of Iran .The small age animals more susceptible to infection through bits of ticks , because innate immunity protect it until 1-6 months but if infected may be due to transmission via the placenta .

The prevalence of *Theileria spp* in camels microscopically according to sex of animals revealed that the highest rate of infected females (69/125) (55.2%) while the lowest rate was in males (24/75) (32%) of total 200 samples. There was significant difference at $p <0.05$. The study results agreed with (24). But disagree with (4) recorded (9/113)(8%) and (23) recorded (27/90)(30%) in males camels. The reason is that the transmission of the disease is one, it's tick of genus *Hyalomma* and that both males and females graze in the same place and when the proportion of female infection is higher due to the number of samples as the number of females in this study more than males because they are used for slaughter. While according to the age number of infected camels less than (<1)year (18/50)(36%)and number of infected camels more than(>1)year (75/150)(50%). There are no significant difference at $p < 0.05$ the results agree with (11,27). But dis agree with (26) who recorded (22/24)(92%) in cattle aged (≥6) years in Northern Tunisia and (23) which recorded infection in differ stage of animals ages. This variations may ascribe to different reasons, including location, population density of animals, environment condition and hygienic measures. furthermore, time of study, the ecological , climatic factors and number of samples. Cases of young age might be under stress because they were weakness and emaciation and more susceptible to

infection .Also recorded mix infection between *Theileria* and *Babesia* with high percentage (23.75%) of the result similar with (24). The reason of occurrence mixed infections in the same animal probably trigger the low health condition of the affected animal, stress creating from chronic diseases, malnutrition. In addition to exposure animals to blood sucking insects and ticks that transmission to blood parasites and also bad hygiene measures.

The Prevalence of Babesiosis and Theileriosis camels using PCR

Molecular methods are more sensitive and specific diagnostic tools PCR done depend on specific primers and positive samples microscopically with show can be infected camels by *B.bovis* ,*B.begimina* , *Theileria spp* and *T. annulata* , also many researcher diagnosed *Babesia spp* by PCR as (13,17)with simple differ in percentages . This differentiations in percentages may be detection of *Babesia spp* occur during the early stage of the disease and carrier animals cases usually difficult to determine the parasite because parasitemia is low . In addition to not all positive samples microscopically for *Babesia spp* diagnosed by PCR technique and many researcher recorded *Theileria spp* like (2,17) they recorded possibility of infection of camels and other domestic animals more than species of *Theileria* , however, found a difference in the proportions of the results, it is due to primers , method and type of PCR Technique .The difference between the results of microscopy and molecular examination is due to the fact that in most cases chronic infections and the amount of DNA were low, so the difference in results

Conclusions and Recommendations

The results showed that (*Babesia spp* and *Theileria spp*) were distributed in camels in Al-Diwaniyah provinces according to Giemsa stain method and PCR technique. Molecular and phylogenetic diagnostics are more accurate in determining the species of parasites. The scientific methods that would develop the production of livestock to raise the economic level of the country through the production of vaccines and the use of preventive treatments to prevent the spread of these diseases. For new research studies are directed to know if these parasites have the potential transmission to humans and detect the pathogenic effects on humans because farmers and owners are in continue contact with the animal.

Reference

1. FAO (Food and Agriculture Organization of the United Nations FAOSTAT) (2014): Food and agriculture organization of the united nations statistics division. Rome, Italy: FAO. Available from: http://faostat3.fao.org, (visited on 2016/07/08).

2. CAO, S; Zhang,S; Jia, L; Xue, S; Yu, L; Ketsarin ,K and *et al* (2013). Molecular Detection of *Theileria* Species in Sheep from Northern China. doi: 10.1292/jvms.13-0028; *J. Vet. Med. Sci.* 75(9): 1227–1230.

3. Swelum, A.; Ismael, AA.; Khalaf, AB. and Abouheif, MA. (2014): Clinical and laboratory findings associated with naturally occurring babesiosis in dromedary camels. *Bull Vet. Inst. Pulawy* 58(2): 229-233.

4. Joshua, K.; Turaki, A.U.; Egwu, G.O.; Mani, A.U.; Saidu, M.K.; Abdullahi, J.G. and Kumshe, H.A. (2008): Haemoparasites of camels (*Camelus dromedarius*) in Maiduguri, Nigeria. *Animal Res. Inter.,* 5(2): 838-839.

5. Barghash, SM.; Abou El-Naga, TR.; El-Sherbeny, EA. and Darwish, AM. (2014): Prevalence of

Trypanosoma evansi in Maghrabi Camels (*Camelus dromedarius*) in Northern-West Coast, Egypt using Molecular and Parasitological Methods. *Acta Parasitol Glob* 5: 125-132.

6. Eyob, E. and Matios, L. (2013): Review on camel trypanosomosis (surra) due to *Trypanosoma evansi*: Epidemiology and host response. *J. Vet. Med. and Animal Health,* 5(12): 334-343.

7. Gutierrez, C.; Corbera, JA.; Juste, MC.; Doreste, F. and Morales, I. (2005): An outbreak of abortions and high neonatal mortality associated with Trypanosoma evansi infection in dromedary camels in the Canary Islands. *Vet. Parasitol* 130: 163-168.

8. Salim, A. Y.; Telmadarraiy, Z.; Vatandoost, H.; Chinikar, S.; Oshaghi, M.; Moradi, M.; *et al.* (2010):Hard ticks on domestic ruminants and their seasonal population dynamics in Yazd Province, Iran. *Iran J. Arthropod Borne Dis.;*4(1):66–71.

9. Ismeal, A.B.; Swelum, A.A.; Khalaf, A.F. and Abouheif, M.A. (2014):Clinical haemtological and biochemical alterations association with an outbreak of theileriois in dromedaries (*Camelus dromedaries*) in Saudi Arabia . *Pak. Vet. J.* 34(2): 209-213.

10. Sazmand, A.; Eigner, B.; Mirzaei, M.; Hekmatimoghaddam, S.; Harl, J.; Duscher, GG.; Fuehrer, H-P. and Joachim, A. (2016): Molecular identification of hemoprotozoan parasites in camels (*Camelus dromedarius*) of Iran. *Iranian J. of Parasitol.,* 11(4): 568–573.

11. Hamed, M.I.; Zaitoun, A.M.A.; El-Allawy, T.A.A. and Mourad, M.I. (2011): Investigation of *Theileria camelensis* in camels infested by *Hyalomma dromedarii* ticks in upper Egypt. *J. of Advanced Vet. Research* 1:4-7.

12. Alim, MA.; Das, S.; Roy, K.; Masuduzzaman, M.; Sikder, S.; Hassan, MM.; Siddiki, AZ. and Hossain, MA. (2012): Prevalence of hemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. *Pak Vet. J.,* 32: 221-224.

13. Qablan, M.; Sloboda, M.; Jirku, M.; Dwairi, S.; Sami Amr, Z.; Horin, P.; Lukes, J. and Modry, D. (2012): Quest for the piroplasms in camels: Identification of *Theileria equi* and *Babesia caballi* in Jordanian dromedaries by PCR. *Vet. Parasitol.,* 186, 456-460.

14. Abd-Elmaleck, BS.; Abed, GH. and Mandourt, AM. (2014): Some Protozoan Parasites Infecting Blood of Camels (*Camelus dromedarius*) at Assiut Locality, Upper Egypt. *J Bacteriol Parasitol* 5: 184.

15. Jasim, HJ.; Azzal, GY. and Othman, RM. (2015): Conventional and molecular detection of *Babesia caballi* and *Theileria equi* parasites in infected camels in south of Iraq. *Bas. J. Vet. Res.;* 14(2): 110-121.

16. Ganjali T. A. (2016): Phylogenetic analysis of camel piroplasmids in Iran based on 18s rRNA gene. MSc. Dissertation, Shahid Chamran University of Ahvaz, [in Persian].

17. El-Naga, T.R.A. and Barghash, S.M. (2016): Blood Parasites in Camels (*Camelus dromedarius*) in Northern West Coast of Egypt. *J. of Bacteriol. and Parasitol.,* 7(1): 1-7.

18. Bilgic, H. B.; Karagenç, T. ; Shiels, B. ;Tait, A. ; Eren, H. *et al.* (2010). Evaluation of cytochrome b as a sensitive target for PCR based detection of *T. annulata* carrier animals. Vet Parasitol 174: 341-347.

19. Leech, N. L.; Barrett, K.C. and Morgan, G.A. (2011): IBM SPSS for Intermediate Statistics. 4th edn. Taylor and Francis Group . LLC. USA.

20. Faham, K.; Doosti, A.; Koohi, A.; Chehelgerdi, M.; Mokhtari-Farsani,

A. and Chengula, A.A. (2015): Determination of the presence of Babesia DNA in blood samples of cattle, camel and sheep in Iran by PCR. *Arch. Biol. Sci., Belgrade*, 67 (1), 83-90.

21. Ibrahim, AM.; Hassan, AA. and Nyingilili, HS. (2017): Microscopic and Molecular Detection of Camel Piroplasmosis in Gadarif State, Sudan. Veterinary Medicine International, *Vet. Med. Intern.*; 9345231: 5.

22. Abdalla, M. I.; Ahmed, A.H.K. and Hamisi, S. N. (2017). Microscopic and Molecular Detection of Camel Anaplasmosis and Piroplasmosis in Banadir region, Somalia. *J Vet Sci Res*, 2(1): 000127.

23. Al-Amery, A.M.; Faraj, A.A. and Majeed, S.A. (2017): Detection of haemoprotozoa in camel in AL-Najaf province, Iraq. Department of Parasitology, College of Veterinary Medicine, University of Baghdad/Iraq . IJABR.7 (2): 238-241.

24. Al-Khaledi, M.J. (2008): Epidemiological study of (Theileriosis, Babesiosis and Anaplasmosis) in cattle of Al-Qadisiya province . M.S. thesis. Vet. Med. College Baghdad Uni. In Arabic.

25. Ziapour, S.P.; Esfandiari, B. and Youssefi, M.R. (2011): Study of The Prevalence of Babesiosis in Domesticated Animals with Suspected Signs in Mazandaran Province ,North of Iran During 2008. *J. of anim. and Vet. Adv.;* 10 (6): 712-714.

26. Sallemi, S.; Rjeibi, M.R.; Rouatbi, A.; Amairia, Ben Said; Mandha, K.K.; Gharbi, M. (2017): Molecular prevalence and phylogenetic analysis of *Theileria annulata* and *Trypanosoma evansi* in cattle in Northern Tunisia. *Vet. Med. and Sci.* ;DOI: 10.1002/vms3.79.

27. Elnga, T.R.; Abd El-Baky, S.M.M. and Abdou, T.A. El-Fayoumy, M.M.; Abou (2005): Prevalence and of camel theileriosis and its vector tick in North Coast of Egypt. *J. of the Egyp. Vet. Med. Assoc*. 65, 291-302.