Plant Archives Volume 20 No. 2, 2020 pp. 7175-7178 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

MOLECULAR DIAGNOSIS OF *THEILERIA* SPP. AND *BABESIA* SPP. INFECTIONS IN *CAMELUS DROMEDARIUS* IN AL-NAJAF AL-ASHRAF PROVINCE, IRAQ

Maytham Askar Alwan Al-Shabbani[1], Ihsan Khudhair Abbas Al Kardhi[2]* and Kifah Fadhil Hassoon Al-Shabaa[1]

[1]Microbiology Department, Faculty of Veterinary Medicine, University of Kufa, Iraq.
[2*]Department of Basic Medical Science, Faculty of Nursing, University of Al-Qadisiyah, Iraq.

Abstract

The present study was goal to confirm the presence of piroplasmosis in venous blood of *Camelus dromedaries* camel from the slaughtered at the main slaughterhouse of Al-Najaf abattoir at AL-Najaf Al-Ashraf province using microscopically (blood film) and molecular technique (PCR). A total of 94male camels were blood collected for microscopic examination and PCR assay to detect the most common of piroplasma parasites which include *Thereria* spp. and *Babasia* spp. the study was beginning from October 2019 till the end of March 2020. Clinical signs and symptoms were recorded in camels,the prevalence rates using microscopic examination and molecular assay were 8.5 % and 18 %, respectively. The state of being prevalent rates was varied significantly between the clinical signs ($P<0.05$) and their causing agents "Theileriosis and Babesiosis", except for one clinical sign oculo-nasal discharge, no significant differences were reported ($P> 0.05$). In conclusion: Polymerase chain reaction test was showed a higher sensitivity than microscopic examination, and a significant association of some clinical signs with piroplasma infection in dromedary camels in Iraq.

Key words: Theileriosis and Babesiosis**,** *Camelus dromedaries*, PCR

Introduction

Babesia are intraerythrocytic organisms of domestic animals and are the cause of anaemia and abnormal high concentrations of hemoglobin urine haemoglobinuria (Zintl *et al.,* 2003). They are transported by ticks in which the protozoa pass through the egg, from one generation to another. The disease, babesiosis, is particularly severe in naive animals that are introduced to endemic areas and is a major constraint on Livestock farming in various countries in the world (Irwin, 2009).The most widespread and pathogenic species in camels is the large *Babesia canis* found in mainland Europe, Africa, Asia and the Americas. *Rhipicephalus sanguineus* is the principal vector, in which transovarian and transtadial transmission occurs.

The diseases caused by many species of theleria are a serious impediment to livestock development in Asia, Africa and the Middle East. Tick-borne parasites undergo frequent schizophrenia, eventually leading to the release of small mirozoite cells that invade red cells into piroplasms (Morrison *et al.,* 2015). *Theleria* spp. are widely distributed in camels in several very large landmasses e.g. Europe, Asia and Australia have a diverse range of tick carriers and are associated with a parasite agent that ranges from mild clinical cases to deadly cases (Qablan *et al.,* 2012). Very few studies about piroplasma parasite infections on camels have recently been found in the one-humped camel such as Egypt (Abd-Elmaleck *et al.,* 2014) and Iran (Branch *et al.,* 2015).

Serious economic losses causing by Theileriosis and Babesiosis still remained to be a most significant threats to camel livestock industry in Iraq, where ticks and flies played a large role in the spread of the disease, the emergence of Camel thelariais and babesiosis poses a serious health effect to animal in highly endemic areas, this parasites can causes a zoonotic disease known as Theileriasis and Babesiosis, Polymerase chain reaction assay have proven to be a very specific and detectible for *detection of Thelaria* and *Babesia* in the venous blood of *Camelus dromedarius*, one-humped camel. The goal of the present study was planned to confirm the

***Author for correspondence :** E-mail : ihsan.khudhair@qu.edu.iq

presence of *Theleria* spp. and *Babesia* sppin camels from the slaughtered at the main slaughterhouse of AlNajaf abattoirat AL-NajafAl-Ashraf province using microscopically (blood film) and molecular technique (PCR).

Materials and Methods

Collection of camel blood samples

A ninety four blood samples were collected from venous blood from the jugular vein of Camel in slaughter house in al Al-Najaf governorate by tubes containing anticoagulant agents, the samples including different ages 16 sample were recorded as <4years old and 78 samples were recorded as >4 years old.

Animals of slaughter house (n = 94) has recently noticed clinical examination of piroplasms infestations and a history of tick infestation, some of them typically have abnormal mucous membrane color, anorexia, increased respiratory rate tachypnea, discolored urine, as well as pertaining of eyes and nose oculo-nasal discharge. Some rare cases showed superficial lymph nodes enlargement; while the other apparently have healthy upon clinical examination, all samples collection was obtained from males of Camel at period beginning from October 2019 to the end of March 2020, then the samples directly transport in ice box into molecular laboratory college of veterinary medicine university of Kufa to performing direct smear examination and molecular assays.

Microscopic Examination

The blood smears were performed through fixed it in 99% methanol for five minutes and quickly stained with Giemsa stain 10% solution (RXMarine International, India) for thirty minutes and directly examination under microscopic apparatus by oil immersion at 100X magnification power.

Blood DNA Extraction

Genomic DNA from blood samples were extracted by using High Pure PCR Template Preparation Kit and done according to company instructions, the Blood and tissue cells were lysed special Lysis Buffer and Proteinase K in short incubation with the presence of a chaotropic salt such as guanidine-HCl. The DNA extraction was read using Thermo Scientific NanoDrop spectrophotometers, the ratio at 260/280nm absorbance was used to assess the purity of nucleic acids, and then kept at deep freezer for molecular assay.

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) amplification was performed with two sets of oligonuclotied sequences primers table 1 which used to detect *Theileria* spp. and *Babesia* sp. protozoa with two amplicon size 694 bp and 383 bp, respectively, These primers were originate from conserved regions of 18S rRNA genes of both parasites in blood of camel, the method was accomplished according to procedure (Shayan and Rahbari, 2005), the oligonuclotied sequences primers in this study were designed in by using Genbank data base NCBI , primers sequences were obtained from company (Macrogen company, Korea) as table 1.

PCR master mix preparation

AccuPower PCR PreMix Kit PCR total volume master mix was prepared according to company instructions as following mix of 20 micoliter volume: 5µl of NA target, 1.5 µl for 10 picomoles forward primer, 1.5 µl for 10 picomoles reverse primer,12 µl of nuclease free water.

Then, all the PCR tubes centerfuged into vortex centrifuge for 2 minutes. Then transferred into thermocycler (MJ-Mini BioRad. USA), the PCR Thermocycler Condition was recorded as 95°C initial Denaturation for 5 minutes, followed by 30 cycles (95°C for 30 seconds, 58°C for 30 seconds and 72°C for 1 minute) and finally, the extension step was recorded at 72°C for 5 minutes. PCR amplicons (694 bp and 383 bp) were examined by agarose 1.5% gel electrophoresis, stained with fluorescent tag "Ethidium bromide" nucleic acid stain and the results were showed by UV transilluminator.

Results and Discussion

In this study, microscopic and molecular screening techniques were used to test the blood of Arabian dromedary camels (*Camelus dromedarius*) for protozoan parasites which causes (Theileriosis and Babesiosis). The taxonomic status of these species remains unclear due to a lack of molecular characterization and experimental infection (Sazmand *et al.,* 2016), therefore, our results considered that the study be exclusively at the species level, not the genus of parasites. In current study, Housekeepinggene-diagnosed primers were used to identify 18rRNA of these parasites in camel blood, these parasites were detected in blood of camels by using both

Table 1:

PCR product	Sequence		oligonuclotied primers
694bp	ATTGGAGGGCAAGTCTGGTG	F	18SrRNA gene
	TGCACCACCACCCAAAGAAT	R	*Theileria* spp.
383bp	AGGAATTGACGGAAGGGCAC	F	18SrRNA gene
	CTAGTGATTACCCGCGCCTT	R	*Babesia* sp.

microscopy and PCR, the result was recorded as (8.5% and18 %) for microscopic and PCR, respectively. Modified Giemsa stain of thin blood smears showed different morphological and developmental features piroplasms in red blood cell by using light microscope, some of samples were showed comma and signet-ring with large vaculae ranged from 0.5 to1.5 µm in diameter shaped as intra-cytoplasmic bodied which was seems to be *Theileria*, some of them have tetrad form, pairs or ameboid aligned shape which was seems to be *Babasia* spp. (Abd-Elmalek *et al.,* 2016), PCR Reaction was used to examine piroplasma infection in camel that was slaughtered after DNA was extracted from its blood, the result showed a 694 base pair size of PCR product belongs to *Theileria* spp. Fig. 1 and a 383bp PCR product sizebelongs to *Babesia* spp. Fig. 2.

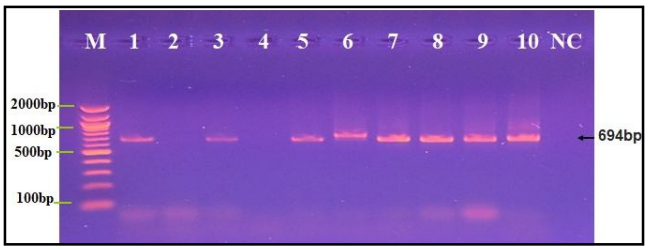

Fig. 1: Agarose gel electrophoresis image of 18SrRNA gene *Theileria* spp.of Camel, 1.0% TBE agarose gel ; M lane : DNA marker (100 to2000 bp), Lane1 to Lane 10 were showed *Theileria* spp. in venous blood of Camel with 694bp of product size , Non template negative control in the Lane 2.

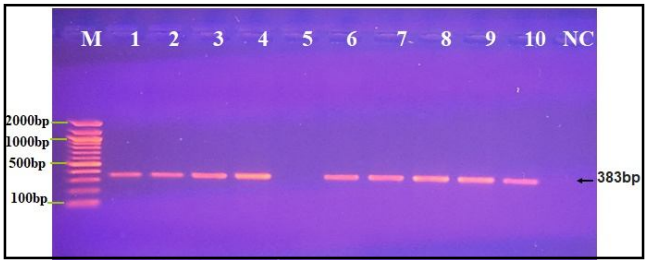

Fig. 2: Agarose gel electrophoresis image of 18SrRNA gene *Theileria* spp. of Camel, 1.0% TBE agarose gel ; M lane : DNA marker (100 to2000 bp), Lane1 to Lane 10 were showed *Babesia* spp. in venous blood of Camel with 383 bp of product size , Non template negative control in the Lane 5.

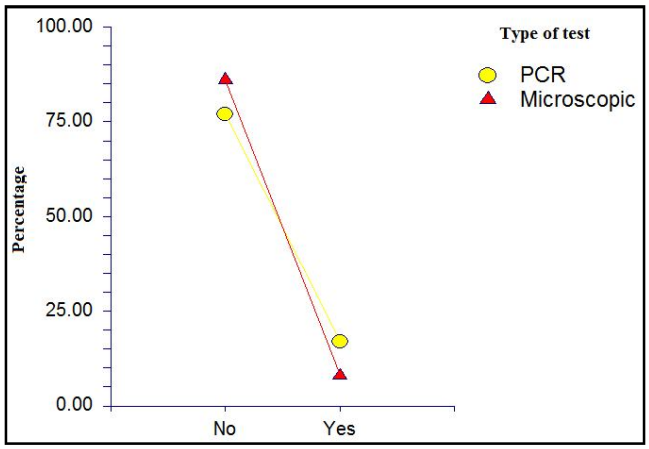

Fig. 3: Prevalence of piroplasm infestation in blood of Arabian camel by using two techniques; PCR molecular assay and Microscopic examination.

PCR assay was found more prevalence to detect both *Babesia* spp. and *Theleria* spp. infestation in camel rather than Microscopic examination Fig. 3, Noaman, 2014 was found among 52 samples, microscopic examination have 100% and 70.83% specificity and sensitivity and rather this ratio was different from PCR assay. The high prevalence of parasites disease observed in the current study may be attributed to the high availability of tick carriers, because the hot and humid environment is very favorable for ticks and ultimately for the survival of the Piroplasma (Chauhan *et al.,* 2015). (Ranjbar, & Afshari, 2009) was showed a prevalence rates in Iranian camel have been reported as 19.47% of *Theleria* spp. In Iran.

Table 2 showed the result of most common of piroplasma infection in age more than 4 years old 62.2% with 95% confidence interval (35.4 to 84.4), it is different from other age group less than 4 years old have percentage 8.9% and 95% confidence interval as (3.6 to 17.6), this differences was significant (P<0.05) increase in age more than 4 years, the results was agreement with (Abaker *et al.,* 2017) who was found the highest prevalence rate was 18.6% among cattle from 1 to 3 years, and the lowest prevalence was 0% among cattle

Table 2:

Variable names	Category	Binomial Proportion (%)	95% CI	x^2 WithDF=1	P value
Age	< 4 Year (78)	8.9 (7/78)	3.6 to 17.6	14	0.008
	> 4 Year (16)	62.5 (10/16)	35.4 to 84.4		
Ticks present	Yes (28)	39.2 (11/28)	21.5 to 59.4	7.7	0.005
	No (66)	9 (6/66)	3.4 to 18.7		
History of Discolored urine	Yes (15)	66.6 (10/15)	38.3 to 88.1	15.1	0.000
	No (79)	8.8 (7/79)	3.6 to 17.6		
Oculo-nasal discharge	Yes (22)	31.8 (7/22)	13.8 to 54.8	2.35	0.12ns
	No (72)	13.8 (10/72)	6.8 to 24		
Enlarged superficial lymph nodes	Yes (24)	54.1 (13/24)	32.8 to 74.4	16.8	0.003
	No (70)	5.7 (4/70)	1.5 to 13.9		

under one year old. On the other hand, the most common cases of parasite infestation were recorded in animals have ticker servoir, one of the most studies have illustrated that protozoan parasites; *Theileria* spp. and *Babesia* spp. were mainly vertical transmitted by reservoir ticks and phylogenetically related to zoonotic species (Iori *et al.,* 2010), Piroplasma spp. can be able to infect RBCs and/ or WBCs of camels (Hamed *et al.,* 2011), in general; *Theileria* spp. and *Babesia* spp. might mature in unattached ticks and can enter the host right after tick attaches (Iori *et al.,* 2010).

x^2 Chisquare test , DF: Degree of freedom, CI: confidence intervals

The study proved that there are cases occurring in camels who suffer from discolored urine , two thirds of the total number (10/15) of camels suffered from hematuria, the prevalence of parasite infestation 66.6% with confidence interval 38.3 to 88.1%, This significant difference ($P< 0.05$) with affected camels without hematuria 8.8% is due to piroplasma can cause hemolytic anemia ,this type of anemia can lead to jaundice yellow-colored skin of camels and red/coffee-colored urine, this case was clarified by (Saini, & Sankhala, (2015) when he studied the relationship of disease with discolored red urine in cattle, The current study showed that there is no significant relationship ($P> 0.05$) between camels that have oculo-nasal discharge with other camels that do not have oculo-nasal discharge, this study was disagreement with (El-Ashker *et al.,* 2015) who was found significant clinical signs e.g. oculo-nasal discharge & hemoglobinuria and the detection of their causing agents Babesiosis and Theileriosis in cattle. Superficial lymph node is characterized by their dromedary lobule size and appearance our study was found that lymph nodes were elongated , ovoid, flattened, rounded or triangular in some cases, this study was adopted by the (Gavrylin *et al.,* 2014) in measuring the enlarged lymph nodes in the camel. A total of 24 dromedary camels that have been slaughter, 13 of them (54.1%) have lymph nodes enlargement with confidence interval ranged (32.8 to 74.4%), In conclusion our result found significant association of some clinical signs with piroplasma infection in dromedary camels in Iraq, also the molecular assay is more reliable than microscopic examination for detection of these parasites.

References

Abaker, I.A., D.A. Salih, L.M. El Haj, R.E. Ahmed, M.M. Osman and A.M. Ali (2017). Prevalence of *Theileria annulata* in dairy cattle in Nyala, South Darfur State, Sudan. *Veterinary world,* **10(12):** 1475.þ

Abd-Elmaleck, B.S., G.H. Abed and A.M. Mandourt (2014). Some protozoan parasites infecting blood of camels (*Camelus dromedarius*) at Assiut locality, Upper Egypt. *J. Bacteriol. Parasitol,* **5(2):** 1-6.þ

Abd-Elmalek, B.S., G.H. Abed and A.M. Mandour (2016). Babesia cameli as a New Species infecting Camels (Camelus dromedarius) at Assiut Locality. *J. Diabetes Metab.,* **7(700):** 2.þ

Branch, S. (2015). Determination Of The Presence Of Babesia DNA In Blood Samples Of Cattle, Camel And Sheep In Iran By PCR. *Archives of Biological Sciences,* **67(1):** 83–90.

Chauhan, H.C., B.K. Patel, A.G. Bhagat, M.V. Patel, S.I. Patel, S.H. Raval and B.S. Chandel (2015). Comparison of molecular and microscopic technique for detection of *Theileria annulata* from the field cases of cattle. *Veterinary world,* **8(11):** 1370.þ

Divergens, a bovine blood parasite of veterinary and zoonotic mportance. *Clinical microbiology reviews,* **16(4):** 622-636.þ

El-Ashker, M., H. Hotzel, M. Gwida, M. El-Beskawy, C. Silaghi and H. Tomaso (2015). Molecular biological identification of *Babesia, Theileria* and *Anaplasma* species in cattle in Egypt using PCR assays, gene sequence analysis and a novel DNA microarray. *Veterinary parasitology,* **207(3-4):** 329-334.þ

Gavrylin, P., D. Rahmoun and M. Lieshchova (2014). Anatomo-topographic features of lymph nodes in the dromedary (*Camelus dromedarius*). **2(1):** 26-31.þ.

Hamed, M.I., A.M. Zaitoun, T.A. El-Allawy and M.I. Mourad (2011). Investigation of *Theileria camelensis* in camels infested by *Hyalomma dromedarii* ticks in Upper Egypt. *Journal of Advanced Veterinary Research,* 1(1), 4-7.þ

Iori, A., S. Gabrielli, P. Calderini, A. Moretti, M. Pietrobelli, M.P. Tampieri and G. Cancrini (2010). Tick reservoirs for piroplasms in central and northern Italy. *Veterinary parasitology,* **170(3-4):** 291-296.þ

Irwin, P.J. (2009). Canine babesiosis: from molecular taxonomy to control. *Parasites & vectors,* **2(S1):** S4.þ

Morrison, W.I. (2015). The aetiology, pathogenesis and control of theileriosis in domestic animals. *Rev. Sci. Tech.,* **34(2):** 599-611.þ

Noaman, V. (2014). Comparison of molecular and microscopic technique for detection of Theileria spp. In carrier cattle. *Journal of parasitic diseases,* **38(1):** 64-67.þ

Qablan, M.A., M. Sloboda, M. Jirkù, M. Oborník, S. Dwairi, Z.S. Amr and Modrý (2012). Quest for the piroplasms in camels: identification of *Theileria equi* and *Babesia caballi* in Jordanian dromedaries by PCR. *Veterinary Parasitology,* **186(3-4):** 456-460.þ

Ranjbar, B.S. and M.A. Afshari (2009). Study on the prevalence of blood parasites in camels of Zabol in 2008.þ

Saini, R.K. and L.N. Sankhala (2015). Babesiosis: a case report in cattle. *International Journal of Science, Environment, and Technology,* **4:** 847-849.þ

Sazmand, A., B. Eigner, M. Mirzaei, S.H. Hekmatimoghaddam, J. Harl, G.G. Duscher and A. Joachim (2016). Molecular identification of hemoprotozoan parasites in camels (*Camelus dromedarius*) of Iran. *Iranian journal of parasitology,* **11(4):** 568.þ

Shayan, P. and S. Rahbari (2005). Simultaneous differentiation between *Theileria* spp. And *Babesia* spp. On stained blood smear using PCR. *Parasitol Res.,* **97:** 281-286.