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COMPARISON OF THE EFFICACY OF DIFFERENT METHODS IN THE DIAGNOSIS OF BABESIOSIS IN CAMELS(*Camelusdromedarius*) IN AL-NAJAF AL-ASHRAF PROVINCE-IRAQ OF NAJAF - IRAQ

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ABSTRACT

This study was designed to evaluate the effectiveness of microscopy compared to molecular detection of babesiosis infection in camels(*Camelus dromedarius*) in AL- Najaf AL-Ashraf province-Iraq, started from November 2017 to May 2018.The results showed that the total prevalence using via microscopic examination and molecular detection method was 17.5% ,54.16% respectively,however , the rate of females infection was 10% as compared with males 25% by microscopic examination , whereas, the infection rate by using PCR in females and males, 66.66% ,41.66% respectively. The results also showed that the rate of infection in the age of less thana year was 6.66% and 28.33% in age of more than one year by microscopical examinations, While by using the molecular assay the rate of infection in the age of less than a year was 45% and 63.33% in the age of more than one year.It has been conclude that PCR has a high ability to detect babesiosis in camels as compared with microscopic examination.

INTRODUCTION

Babesia species anapicomplexan–hemoprotozoan parasite transmitted by Ixodidae ticks,they refer to that more than 100 species have been phenotypically documented (1,2).This parasite is considered a destructive affecting livestock production (3). And the infection is of global importance which are characterized anemia, icterus, hemoglobin urea, muscles trembling, grinding of teeth(4). The feces are dry and bloody stained, dehydration causes the eye to become

sunken in their sockets, falls of body temperature to a subnormal level before a few hours of death , anemia , anorexia , death of untreated cases (5).

Diagnosis of hemoprotozoanparasite is beneficial in early diagnosis. conventionally, microscopic examination has been considered the "gold standard" for diagnosis piroplasmosis in blood smear in acute cases of infected animals but not in chronic cases(6,7).Therefore,proven efficacy and sensitivity of molecular tests in detectionof *Babesia*sp. (8,9).The aim of this study to evaluate the traditional methods compared to molecular detection for the diagnosis *Babesia* spp. in camels in AL- Najaf AL-Ashraf province-Iraq.

MATERIALS AND METHODS

Blood Samples

120 camel's blood samples (60 females and 60males) of different ages were randomly selected, during the period started from November 2017 to May 2018 in AL-Najaf AL-Ashraf province. Blood samples from slaughtering of animal were collected ,Five milliliter EDTA blood samples were always used for collection of blood.Samples of blood were transferred within ice box to the laboratory of College of Veterinary Medicine University of Baghdad and laboratory of AL-Razi Center for research and medical diagnostic kits until for laboratory teststo conduct the necessary tests to diagnosis of*Babesia*spp.

Slides preparation

Thick and thin blood smears were prepared according to (10).

DNA extraction and PCR assay:-

Oneprimer were used in this study were obtained from IDT company (Bab-spgenes). This primers were prepared according to the information of the company (Table.1).

Primers	**Primer sequence (5′ to 3′)**		**Product size (bp)**	**References**
Bab-sp	F	GTTTCTGCCCCATCAGCTTGAC	400	(11)
	R	CAAGACAAAAGTCTGCTTGAAAC		

Statistical Analysis

The data were subjected to analysis using SAS software (12). The McNemar's test was used to assess the difference between the two tests and the degree of agreement between the microscopic and PCR tests was evaluated using Kappa coefficient. The differences between groups according to sex and age for the microscopic and PCR tests were assessed using Chi-square. $P<0.05$ is considered significant.

RESULTS

Results showed that the overall incidence rate using the microscopic examination and molecular detection method was 17.5% ,54.16% respectively figure (1).The rate of infection in females was 10% andmales 25% by microscopic examination , while the infection rate by using PCR in females and males was41.66%, 66.66% respectively (Table, 1).

The differences in infection rate between two tests were significant($P<0.0001$) according to sex .The agreement between the two tests according to Kappa coefficient was -0.16 which means no agreement was found between two tests.

The differences in the infection rate between males and females were significant ($P=0.03$) according to microscopic test as well as the PCR ($P=0.005$).

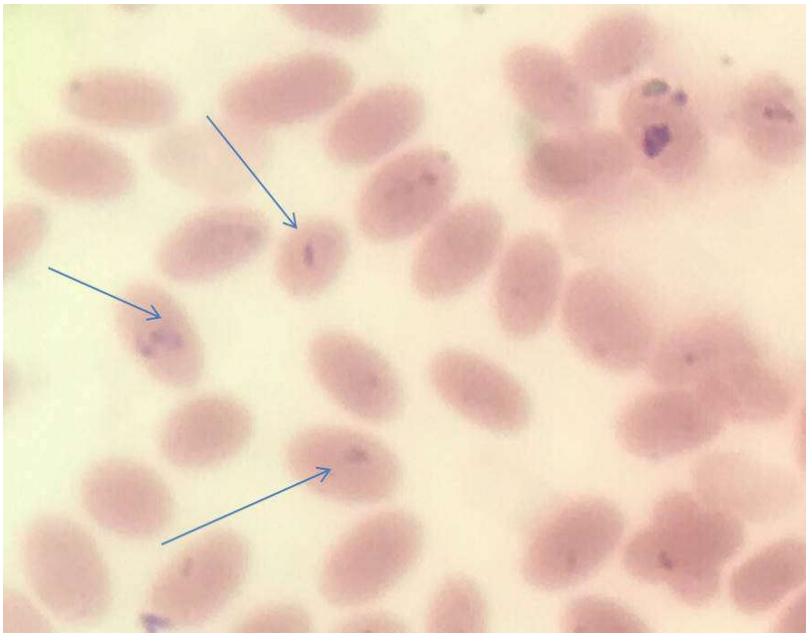

Figure (1): blood smear stained with Giemsastain(10%) show *Babesia spp.*with different shapes in red blood cells of camel(arrows) (X 100).

Table1: The infection rate of *Babeisa*spp. in camels according to sex using microscopic examination and PCR.

Sex	No. of blood Sample	Microscopic Examination No. infected (%)	PCR No. infected (%)	McNemar's value	P
Male	60	6(10)	25(41.66)	22.04	<0.0001
Female	60	15(25)	40(66.66)		
Total	120	21(17.5)	65(54.16)		
Chi-square value		4.67	7.55		
P		0.03	0.005		

Table (1) show that the percentage of infection in theage group≤1 year and ≥1 year was 6.66%, 28.33% respectively by microscopical examination, while by using the molecular assay the rate of infection in females and males was 45%, 63.33% respectively.

The differences in infection rate between two tests were significant(P<0.0001) according to age,the agreement between the two tests according to Kappa coefficient was -0.21 which means no agreement was found between two tests.

The differences in the infection rate between age groups were significant (P=0.001) according to microscopic test as well as the PCR (P=0.04).

Table1: The infection rate of *Babeisa*spp. in camels according to age using microscopic examination and PCR.

Age year	No. of blood Sample	Microscopic Examination No. infected (%)	PCR No. infected (%)	McNemar's value	P
≤1	60	4(6.66)	27(45)	20.83	<0.0001
≥1	60	17(28.33)	38(63.33)		
Total	120	21(17.5)	65(54.16)		
		9.75	4.06		
P		0.001	0.04		

The resultsshowed amplification conditions were optimized for the PCR assay, using specific primers of Bab-sp(400bp), figure (2).

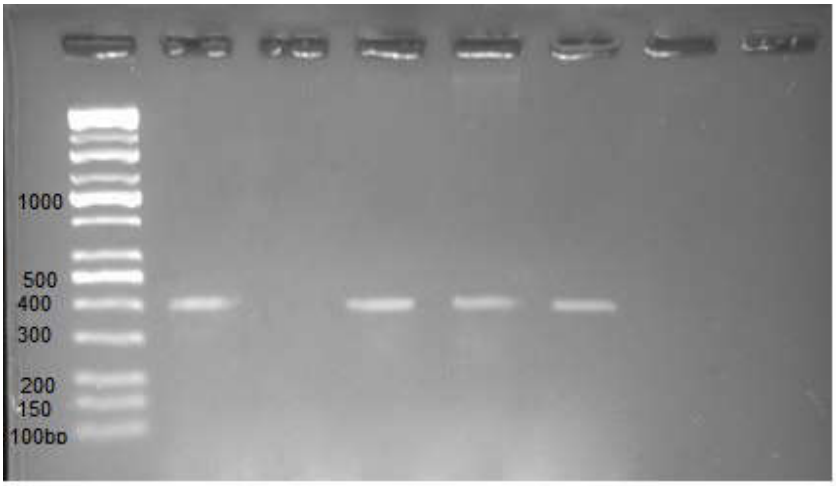

Figure (2): Gel electrophoresis of PCR product of Bab-sp *(*4004bp), for *Babesia spp.*using 2% agarose gel at 6volt /cm for 1 hour. Lane 1- 3,4,5: PCR product positive for *Bab-sp* genes, M: 2000-bp DNA marker.

DISCUSSION

Tick-borne diseases considered as major problems for the health camel in Iraq ,among these diseases, camel babesiosis is the most prevalent and economically important (13). Also, the microscopic diagnosis of babesiosis in many parts of Iraq is done by examination of blood smears .This method is unreliable especially in subclinical infections. To determine the epidemiology agents of babesiosis, sensitive and specific diagnostic methods, such as polymerase chain reaction (PCR), are required to be used in Iraq, which had been developed in order to overcome the problems faced with conventional and serological assay, In addition PCR is a reliable method for diagnosis and epidemiological studies.

Results showed that an overall infection rate with *Babesia* spp. in camel as 17.5% by using microscopic examination , these findings are similarly to previous studies that recorded an overall prevalence of 9.98% for *Babesia* in South Iraq (14), 29%in Pakistan (15) ,13.2%In Saudi Arabia (16) and 6.56% in Iran (11). This variation in the infection rate between the countries may be due to differences in the numbers of examining the animals, survey periods climatic factors also affect the abundance of viable parasitic. In the present study, the incidence of camel babesiosisdiagnosis by PCR (54%) was significantly higher than diagnosed through traditional

examination of .Therefore, DNA amplification tests had higher efficiency than microscopic examination for detection of *Babesia*. The results were in agreement with a previous report about camel babesiosis(17,18).This difference is due to the high sensitivity of the polymerase chain reaction technique compared to the conventional examination, In addition, dependent on the experience of the microscopes (19). On the other hand the microscopic examination not reveal be used to diagnosis the chronic cases due to low number parasite (14).This difference is due to the high sensitivity of the polymerase chain reaction technique compared to the conventional examination and also depends on the experience of the examination and the microscopic examination does not reveal chronic cases .our results, the infection percentage of camel babesiosis by microscopically and molecular methods was significantly higher in age ≥1year. This results agreement with other studies according to the age-related immunity to babesiosis(20,21). In previous observation there was no difference between *Babesia* spp. prevalence in all ages and significant higher Babesia spp. (22). In general, young and adults are susceptible to babesiosis, while in young cattle maternal antibodies persist for the longer period of three months(23). Also,haemoglobin fetal (HbF) found in younganimalswhich is considered one of the high resistance factors against for haempprotozoan parasites.(24).Concerning sex susceptibility to infection, current study showed the high rate of infection of Babesiosis in females may be due to stresses factor causes immune depression such as pregnancy and lactation period were in agreement with(25) .We conclude from this study that the animalreservoirs Increase the risk of spread of the disease in animalsand especially humans, andthis drawsspecial attention. The results indicated a high efficiency of using PCR to detect babesiosis in carrier hostscompared to using conventionalmethods.

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