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Microscopic and molecular detection of *Babesia bovis* and *Babesia bigemina* in female camel from Al-Diwaniyah province, Iraq

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Abstract

This study identified the etiological pathogens responsible or protozoal-like disease conditions in female camels from Al-Diwaniyah Province, Iraq. For this reason, 125 female camels (one blood sample per animal) that showed signs of weakness and pale mucus membranes were considered for the study. The samples of stained blood smears were explored microscopically and via a polymerase chain reaction (PCR) method that the targeted glutamine-dependent carbamoyl phosphate synthase (CPSII) gene for identifying *Babesia bovis* and *18S rRNA* gene for detecting *B. bigemina*. The results of the microscopic technique uncovered the occurrence of *Babesia* spp. in 76 (60.8%) of the examined samples, which encourage the use of PCR to identify the protozoal species. The PCR findings demonstrated that *B. bovis* and *B. bigemina* were detected in 8 (8.9%) and 11 (12.22%), respectively, of the positive microscopic samples. The study findings reveal that weakness and paleness of mucus membranes in camel females can be attributed to the presence of infections by blood protozoa, mainly *Babesia bovis* and *Babesia bigemina*.

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Introduction

Babesiosis is a protozoan parasite carried by vectors that affect the veterinary and medical fields (1). Some hard ticks belonging to the *Hyaloma*, *Rhipicephalus*, and *Dermacentor* families deliver apicomplexan intraerythrocytic protozoa of the genus *Babesia* to a wide range of wild and domestic vertebrate animals, especially to domestic animals such as cows, sheep, goats, dogs, mice, rats, cats and birds, as well as humans, Babesiosis pathogenic agents are different species of the genus *Babesia*. Hemolytic anemia is a common consequence of babesiosis, which has been linked to many Babesial species, particularly *B. bovis* and *B. bigemina* (2). Apicomplexan shapes inside an erythrocyte appear as rings, ovals, maltan crosses, pears, and amoeboid forms with a length of 1-2.5 or 2.5-5 µl (3). For animals, the usual approach to diagnosing babesiosis depends on microscopic parasites methods in Giemsa-staining of venous blood samples and evaluation of clinical symptoms throughout the acute stage of the illness (4). Subclinical babesiosis infections are seen in babesiosis-infected animals throughout their life duration. In rare cases, microscopic examinations fail to find any parasites, resulting in false-negative results (5). This disease is difficult to diagnose based on appearance alone since many parasitic organisms may infect animals. As a result, molecular screening procedures can provide advantages for more precise identification of *Babesia* species and distinction from *Theilera* parasites in a time and cost-effective approach, particularly in parasitemia (6).

This study identified the etiological pathogens responsible or protozoal-like disease conditions in camel females from Al-Diwaniyah Province, Iraq.

Materials and methods

Blood samples

In the present study that was carried out from September, 2017 until the end of March, 2018, 125 female camels (*Camelus dromedaries*) were brought to a slaughterhouse in

Al-Diwaniyah province, Iraq, and showed signs of weakness and pale mucus membranes with other asymptomatic camels were included in the present study. One blood sample per animal was collected from the jugular vein at 5 ml per female. Clean and sterile EDTA-blood-collecting tubes were used and immediately icebox-transported to the Laboratory of Parasitology, the College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah City, Iraq.

Microscopic examination

Thin blood smears stained with Giemsa stain on slides were employed to identify the presence of protozoa based on morphological characteristics as described by Soulsby (7). Briefly, a blood drop was placed on a slide and was spread out on this slide by another slide to make a thin smear. Then, the smear was left on a bench to dry and then to be absolute-methanol-fixed for 5mins, followed by staining with Giemsa stain for 30mins. Later, the slide was water-washed and left to dry. Finally, the slides were examined under a light microscope using an oil immersion lens (7).

Extraction of *Babesia* genomic DNA

The extraction of the *Babesia* genomic DNA was performed using the Column-pure blood Genomic DNA Mini Kit (Applied Biological Materials (Abm, Canada). The extraction procedures were performed per the kit protocol. The DNA was read to understand its purity and concentration using a NanoDrop.

PCR reaction

The primers used in the present study that targeted the CPSII gene for the detection of *B. bovis* and the *18S rRNA* gene for the detection of *B. bigemina* are listed in table 1 (8-10), table 2 (11), table 3 (12) and table 4.

Table 1: Primers used in this study

Gene and *Babesia* species		Sequence (5'-3')	Product size (bp)	Reference
Babesia bovis CPSII	F	TTTGGTATTTGTCTTGGTCAT	446-453	(8)
	R	ACCACTGTAGTCAAACTCACC		
Babesia bigemina 18ribosomal RNA gene	F	TAGTTGTATTTCAGCCTCGCG	689	(9)
	R	AACATCCAAGCAGCTAHTTAG		
	R	TTGCCTTAAACTTCCTTG		

Table 2: The components of monoplex PCR

PCR components	Volume (µl), 25 µl of total
Master mix (2x)	12.5
F Primer	1.25
R Primer	1.25
DNA	5
PCR grade water	5

Table 3: The components of multiplex PCR

PCR components	Volume (µl), 25 µl of total
Master mix (2x)	12.5
F Primer (1)	0.75
R Primer (1)	0.75
Forward Primer (2)	0.75
Reverse Primer (2)	0.75
DNA template	5
PCR grade water	4.5

Table 4: PCR amplification program

Babesia species	Initial Denaturation	Cycle repeats= 40			Final extension
		Denaturation	Annealing	Extension	
Babesia bovis	94°C/280s	94°C/120s	59/60s	72°C/60s	72°C/60s
Babesia bigemina	94°C/280s	94°C/120s	58/60s	72°C/60s	72°C/60s

Agarose gel at 1.5% stained with ethidium bromide was used in electrophoresis. The gel, then, was illuminated under a UV-imager.

Results

The results of the microscopic technique revealed the occurrence of *Babesia* spp. in 76 (60.8%) of the examined stained blood samples (Figure 1), which encourage the use of the PCR to identify the protozoal species.

The PCR findings demonstrated that *B. bovis* (Figure 2) and *B. bigemina* (Figure 3) were detected in 8 (8.9%) and 11 (12.22%), respectively, of the positive microscopic samples.

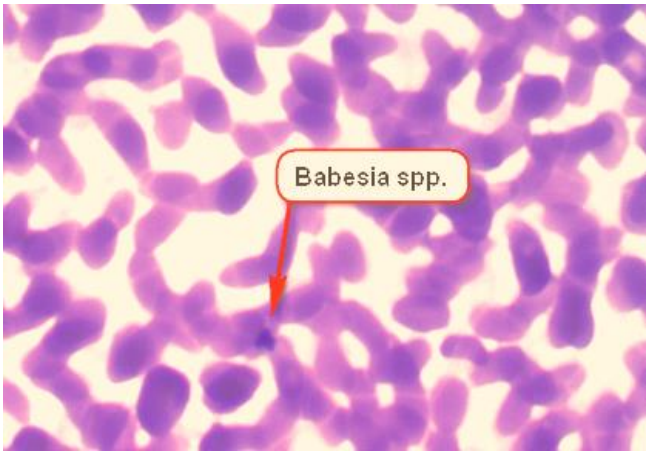

Figure 1: Microscopic image of a blood sample stained with Giemsa stain from a female camel that shows the presence of *Babesia* spp inside an erythrocyte. 100x

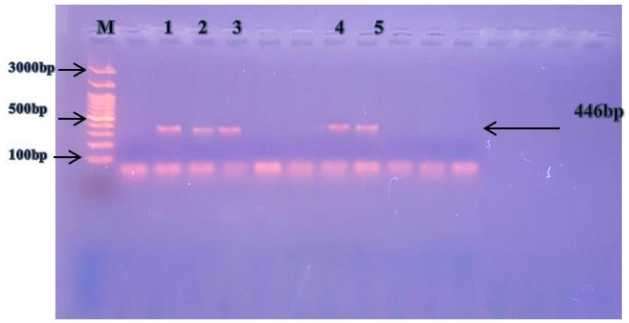

Figure 2: Image of 1.5% agarose gel electrophoresis of blood samples from positive camels that show the presence of *Babesia bovis*. M: PCR ladder (100-3000 bp) and lines; 1 to 5: Some positive blood specimens for *Babesia bovis* at 446bp for the glutamine-dependent carbamoyl phosphate synthase (CPSII) gene.

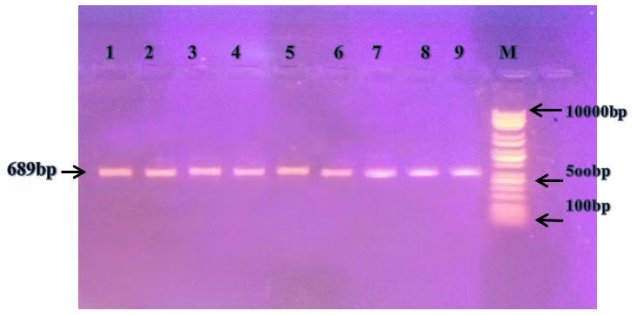

Figure 3: Image of 1.5% agarose gel electrophoresis of blood samples from positive camels that show the presence of *Babesia bigemina*. M: PCR ladder (100-1000bp) and lines; 1 to 9: Some positive blood specimens for *Babesia bigemina* at 689bp for the *18S rRNA* gene.

Discussion

Tick-borne intraerythrocytic parasites, such as *Babesia* species, may cause severe or deadly infections in vertebrates in the late spring and early summer. *Babesia* parasites are not often seen in camels (12). Therefore, *Babesia* infections in camels have been understudied. The current study's clinical findings agree with Swelum *et al.* (13), who detected some clinical findings in camels infected with babesiosis, such as anemia, hemoglobinuria, and jaundice.

The current study's microscopic examination detected the presence of *Babesia* spp. in the blood samples of female camels in a high percentage. Mirahmadi *et al.* (3) detected, using Giemsa staining in a microscopy examination that only 10% of their 140 tested camels appeared to be infected with *Babesia* spp. in Iran. In addition, Ibrahim *et al.* (14) reported the occurrence of the protozoa in 43.6% of their tested camels in Sudan. Many studies have been done on babesiosis in various vertebrate animals. However, there have been few studies on *Babesia* infections in camels from different countries. *Babesia* was found in 3.54% of camels in a microscopy investigation in Zabol, Iran (15). Eight out of 122 camels tested positive for *Babesia* infections in another study conducted in Ahvaz, Iran; however, the species of the infected camels was not determined (16-18).

When it comes to diagnosing *Babesia*, El-Naga and Barghash (8) tested the two approaches and found that PCR had a higher detection rate (18.43%) than Giemsa staining (11.8%). Mirahmadi *et al.* (3) showed that all babesiosis infections were caused by *B. caballi*, which varies from the results of Ibrahim *et al.* (14), who found just one incident of *B. caballi* infection out of 200 afflicted camels. According to a study published in 2015 by Jasim *et al.* (19-21), the percentage of *B. caballi* infection in camels detected by two particular primers was 39.47%, and camels should be regarded as a source of the infection transmission to horses. Also, the spread of *B. caballi* by ticks in camels in Jordan has been documented by Qablan *et al.* (22). This importance of infection in camels encourages the investigation of protozoal infections in camels.

Conclusion

The current study findings reveal that weakness and paleness of mucus membranes in camel females can be attributed to the presence of infections by blood protozoa, mainly *Babesia bovis* and *Babesia bigemina*.

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Conflict of interests

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التحديد المجهري والجزيئي للبابيزيا البقرية والبابيزيا بايجيمينا في إناث الإبل في محافظة الديوانية، العراق

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الخلاصة

أجريت هذه الدراسة للتعرف على العوامل المسببة للحالات المرضية الشبيهة بالأولي التي حدثت في إناث إبل في محافظة الديوانية، العراق. تضمنت الدراسة الحالية ١٢٥ ناقة (عينة دم واحدة لكل حيوان) التي ظهرت عليها علامات الضعف وشحوب الأغشية المخاطية. تم فحص عينات الدم المصبوغة مجهريًا ومن خلال استخدام طريقة تفاعل البلمرة المتسلسل التي استهدفت جين المكون لكاربومايل فوسفات الكلوتامين للكشف عن البابيزيا البقرية وجين الرايبوسومي للكشف عن البابيزيا البايجيمينا. أظهرت نتائج الفحوصات المجهرية عن وجود أنواع البابيزيا في ٧٦ (٦٠,٨٪) من العينات المفحوصة، مما شجع على استخدام تفاعل البلمرة المتسلسل للتعرف على أنواع الأوالي. أظهرت نتائج الفحص على اكتشاف الطفيليان في ٨ (٨,٩٪) و ١١ (١٢,٢٢٪) على التوالي من العينات المجهرية الموجبة. كشفت نتائج الدراسة أن ضعف وشحوب الأغشية المخاطية في إناث الإبل يمكن أن يعزى إلى وجود عدوى بواسطة أوالي الدم، وخاصة البابيزيا البقرية والبابيزيا البايجيمينا.