

Molecular detection of *Babesia bovis* and *Babesia bigemina* in Cattle in
Al-Qadisiyah Province

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Summary

This study aims to determine *B. bigemina* and *B. bovis* based on genetic methods . A total of ١٩٢ blood and spleen tissue samples were collected from a live and slaughtered cows from different regions of Al-Qadisiyah province from December ٢٠١٣ to August ٢٠١٤ . RT.PCR technique was used to detect the presence of *B. bigemina* and *B. bovis* with study the effect of animals age and sex in the distribution of infection .

Overall ٤٣.٢٢% (٨٣/١٩٢) of examined cows showed positive infection with Babesiosis. among them ٤٧.٩١ % (٤٦/٩٦) and ٣٨.٥٤%(٣٧/٩٦) were given positive result to *B. bovis* and *B .bigemina* respectively . The highest infections were shown among the adult cow (≥ 1 year) While there were a variation regarding to two species of parasite in infection according to the sex but with non-significant difference ($P \geq 0.05$) .

Key words: Babesiosis, *Babesia bigemina* , *Babesia bovis*, cattle

التحري الجزيئي عن *Babesia bovis* و *Babesia bigemina* في الأبقار في محافظة القادسية

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الخلاصة

أن الهدف من هذه الدراسة هو لتحديد *B. bovis* و *B. bigemina* اعتمادا على الطرق الوراثية. تم جمع ١٩٢ عينة دم وأنسجة طحال من أبقار حية وأبقار مذبوحه من مناطق مختلفه في محافظه القادسية خلال الفتره من شهر كانون الأول ٢٠١٣ ولغاية شهر اب ٢٠١٤ .

استخدمت طريقة تفاعل السلسلة المتبلمرة في الوقت الحقيقي لتحديد وجود *B. bovis* و *B. bigemina* مع دراسة تأثيرات عمر و جنس الحيوان في توزيع الإصابة. أظهرت النتائج ان ٤٣.٢٢% (١٩٢/٨٣) من الأبقار المفحوصة كانت مصابة بداء الكمثریات وظهر من بينها ان ٥٥.٤٢% (٨٣/٤٧) و ٤٤.٥٧% (٨٣/٣٧) كانت مصابة بالنوعين *B. bovis* و *B. bigemina* على التوالي. وقد تركزت الإصابة أكثر في الأعمار الكبيرة (أكثر من سنة واحدة) و كانت هنالك اختلاف في نسب الإصابة بالنسبة للنوعين اعلاه اعتمادا على الجنس ولكن بدون فرق إحصائيا ($p > 0.05$) نستنتج من ذلك ان الأبقار في محافظة القادسية لديها تحمل طبيعي للإصابة بطفيليات *B. bovis* و *B. bigemina* الامر الذي جعلها حاملة للإصابة بصورة دائمية.

الكلمات الافتتاحية: داء الكمثریات, *Babesia bigemina*, *Babesia bovis*, الأبقار

Introduction

Piroplasms are a tick – transmitted parasitic protozoa parasites divided into two genera *Theileria* and *Babesia*. They are the causative agents of Theileriosis and Babesiosis, respectively (١,٢).

Many *Babesia* spp. have been described since Victor Babes who first recognized *Babesia* in the red blood cells of cattle in ١٨٨٨ (٣).

Researchers (٤) to pointed that the species of *B. bovis* and *B. bigemina* affect cattle, and widely important spread in many parts of Asia, Africa, Australia and America because of the presence of the main vector of *Babesia* spp. that represent by *Boophilus microplus*, and is wide spread in the tropics and sub tropics areas.

Cattle between ٣ and ٩ months of age have higher innate resistance to most tick –borne diseases and consequently disease incidence and corresponding mortality are typically lower for this stock class. If a sufficiently high proportion of a herd are consistently exposed to *Babesia* spp. as calves a state of endemic stability may develop in which clinical tick fever is rarely seen (٥).

Babesiosis was recorded in various domestic and wild animals in Iraq , with variation in proportions of infection depended upon factors like age , breed , season and activity of ticks (٦,٧) .

Most of the previous studies depended in detection of parasite upon the microscopical examination, so the aim of this study was to detect the blood parasite (*B. bovis* , *b. bigemina*) genetically in addition to show the effect of age and sex of animals on the disease distribution.

Materials and Methods

This Study was conducted during the period from December ٢٠١٣ to August ٢٠١٤ in different areas of AL-Qadisiyah province . A total of ١٩٢ blood and spleen tissue samples were Collected from cows clinically suspected to be infected with babesiosis.

The examined cows included ١٣٢ males and ٦٠ females where distributed according to the age into three groups involved calves less than or equal to six months (≤ ٦ m) , young cows ranged between six months to one year and adult cows with age of equal or more than one year(≥ ١ y) .

Two – five ml of blood sample were collected directly from the Jugular vein or during the slaughtering of suspected animal and kept in anticoagulant EDTA tubes , in addition to the collection of ٥ – ١٠ grams of spleen tissue in sterile plastic containers . All samples were transferred in cooling conditions to the laboratory of parasitology in Veterinary Medicine Collage in AL-Qadisiyah University to conduct the necessary tests to determine the infection with babesiosis according to the experimental design .DNA extraction from blood

and tissue samples was done by used the Genomic DNA extraction Kit (Bioneer/Korea) according to the manufacturers instruction.

The extracted DNA were tested by RT-PCR method through used the RT-PCR kit (Genkam/Germany) for *B. bovis* and *b. bigemina* , the thermocycler conditions was done according to primer annealing temperature and probe that included one cycle of Pre-Denaturation in 90 °C for 0 min, and 40 cycles of Denaturation in 90 °C for 10 sec, Annealing/Extension in 60 °C for 30 min and Detection (Scan) was 60 °C for 30 min .

Results:

1- Prevalence of Babesiosis in cattle according to real-time PCR test:

The results showed that among (192) babesiosis suspected cases were examined by real – time PCR, 82(42.72%) gave a positive result to babesiosis infection. Among these infected cases and regarding to the species, the results revealed that 47.91%(46/96) from which were infected with *B. bovis* and 38.04%(37/96) with *B. bigemina* (Table 1, Figure 1, 2).

Table (1) : Real – time PCR positive cases of Babesiosis

<i>Babesia</i> Species	Examined No.	Positive No.	%
<i>Babesia bigemina</i>	96	37	38.04 a
<i>Babesia bovis</i>	96	46	47.91 a
Mixed infection	192	10	5.20 b

- Similar letters refer to the non-significant differences among species while different letters refer to significant differences at ($P \leq 0.05$).

٢- Prevalence of Babesiosis cases according to the sample:

Related to the used samples in detected of the parasite species, the results showed that there is a relative highest were recorded in blood samples (٢٤ % , ١٩%) when compare with spleen samples (٢٢% , ١٨%) in both *B. bovis* and *B. bigemina* respectively (Table ٢).

Table (٢):Real – time PCR positive cases according to the sample.

<i>Babesia</i> Species	Examined No.	Positive No.in		positiveNo.in	
		blood	%	spleen	%
<i>Babesia bovis</i>	٩٦	٢٤	٢٥ ^a	٢٢	٢٢.٩١ ^a
<i>Babesia bigemina</i>	٩٦	١٩	١٩.٩٧ ^a	١٨	١٨.٧٥ ^a
Total	١٩٢	٤٣	٢٢.٣٩	٤٠	٢٠.٨٣

- Similar letters refer to the non-significant differences among samples at ($P \geq 0.05$).

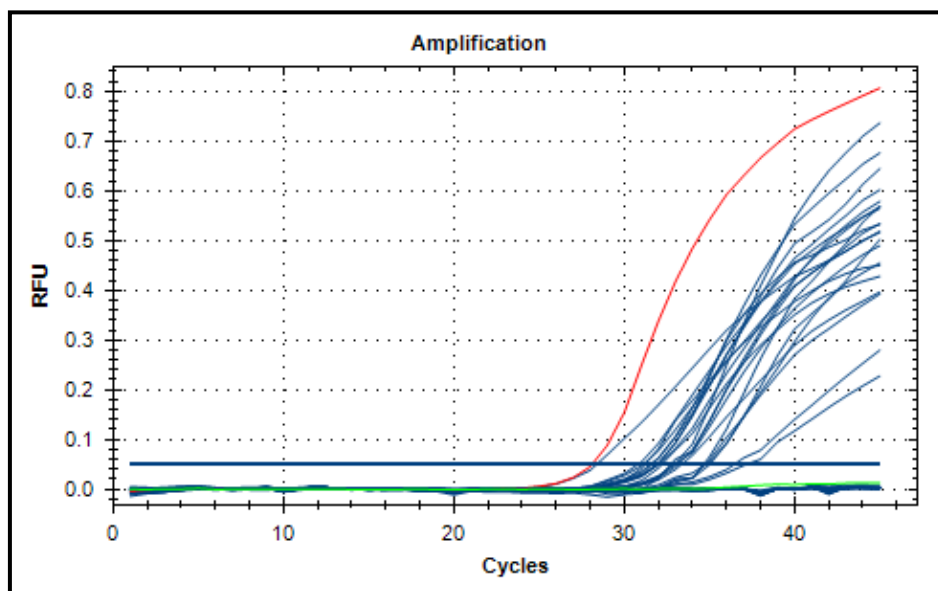


Figure (١): Real-Time PCR amplification plot for *B. bovis* in positive and negative samples

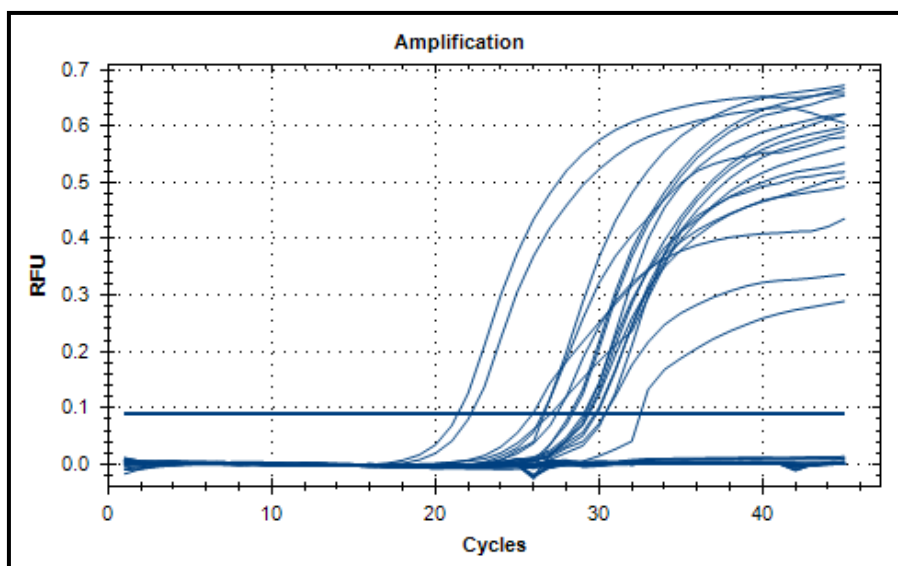


Figure (2): Real-Time PCR amplification plot for *B. bigemina* in positive and negative samples

3. Prevalence of Babesiosis cases according to the Age .

In both species the highest infection (62.5% and 78.12%) were seen among cattle with age of equal or more than 1 year($\geq 1y$) (Table 3).

Table (3) : Real – time PCR positive cases of different species of Babesiosis according to age .

<i>Babesia</i> species	Age	Examined No.	Positive No.	%
<i>Babesia bovis</i>	≤ 6 mns	32	6	18.75 ^a
	6 mns - 1y	32	10	31.25 ^b
	$\geq 1y$	32	25	78.12 ^c
	Total	96	41	42.71
<i>Babesia bigemina</i>	≤ 6 mns	32	6	18.75 ^a
	6 mns - 1y	32	11	34.37 ^b
	$\geq 1y$	32	20	62.5 ^c
	Total	96	37	38.54

- Similar letters refer to the non-significant differences among ages while different letters refer to significant differences at ($P \leq 0.05$).

4- Prevalence of Babesiosis cases according to the sex:

Regarding to the sex the results appeared that the females cows recorded the higher rate (50%) in *B. bovis* infection while the male recorded the higher rate (39.39%) in *B. bigemina* infection (Table 4).

Table (4): Positive cases in Real – time PCR according to animal sex

<i>Babesia</i> species	Sex	Examined No.	Positive No.	%
<i>Babesia bovis</i>	Male	66	31	47.96 ^a
	Female	30	15	50 ^a
	Total	96	46	47.91
<i>Babesia bigemina</i>	Male	66	26	39.39 ^a
	Female	30	11	36.66 ^a
	Total	96	37	38.54

- Similar letters refer to the non-significant differences among sexes at ($P \geq 0.05$).

Discussion

In the current study and according to the RT-PCR test the result showed that 43.22% of suspected cases appeared positively to infection with babesiosis. Regarding to the species of parasite the results showed that the rates of infection with *B. bigemina* and *B. bovis* were 38.54% and 47.91% respectively, while the mixed infection was 0.20%.

Through access to the results of other studies it found that most of them had the lowest results than which came in the current study, as indicated by Abdo-Sakaya (8) from Egypt who said that the rate of infection with *Babesia*

spp. reached 20.33% also scores of (9, 10 and 11) ratios close of approximately 11%. The infection rate in study conducted by Devos and potgieter (12) in France was 20%, as well as the rates were amounted in other studies like (13, 14 and 15), the difference among the current study and other different studies may be attributing to difference in the samples number and type, climate conditions, epidemic of parasite and vector. The prevalence of infection and the occurrence of disease are determined by complex interactions between the bovine host, vector and parasite (16). PCR does not provide information on herd immunity. Tick-borne pathogen densities in carrier animals appear to fluctuate over time and periodically fall below the levels detectable by PCR (17, 18).

This study appeared a mixed infection of two studied species (*B. bigemina* and *B. bovis*) which reached to 0.20%. In Turkey Dumanli and Ozer (19) found a mixed infection between *B. bigemina* and *T. anulata* reached 1.0% in cattle, also Co-infection with *Babesia*, *Theileria* and *Anaplasma* was seen in a Bangladesh by Abdul karim (20), and in Egypt Nayel (15) found mixed infection of *Babesia* and *Theileria* (2.22%)

The present study pointed to that infection with babesiosis according to age revealed high percentage (48.12% and 62.0%) in animals with equal or more than one year, where as the lowest result were recorded in animal with age under six month (≤ 6 mons). The highest rate of infection in adults animal may be due to the chronicity of infection which can easily detectable by real time-PCR method. Results above don't correspond with (21) who recorded rate only 28% in calves and 10% in adult cows.

According to sex the results appeared that non significant differences between males and females in *B. bovis* and *B. bigemina* infections, and this may be due that both sexes subjected to the same condition of rearing like nutrition and climate.

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