Molecular detection of *Babesia bovis* in cattle in Al-Qadisiyah province

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Summary

This study aim to determine *Babesia bovis* infection in cattle based on genetic methods. A total of 96 blood samples were collected from alive and slaughtered cattle from different areas in addition to the abattoir of Al-Qadisiyah province from December 2013 to August 2014. Real time polymerase chain reaction (RT.PCR) technique was used to detect the presence of the protozoan with the effect of animal's age and sex in the infection rate 47.91 % (46/96) of examined cattle were given positive result to *B. bovis* infection. The highest infections were shown among the adult cattle (≥1 year), while there was non-significant difference (P>0.05) in the infection rate according to the sex. So the most cattle in Al-Qadisiyah province appear to be bearing the infection predominantly as a carrier hosts.

Keywords: Babesiosis, *Babesia bovis*, Cattle.

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 (1 and 2). Many *Babesia* spp. have been described since Victor Babes who first recognized *Babesia* in the red blood cells of cattle in 1888 (3). Bock *et al*. (4) 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 represents by *Boophilus microplus*, and is wide spread in the tropics and sub tropics areas.

Cattle between 3 and 9 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 *Basbesia* spp. as calves a state of endemic stability may develop in which clinical tick fever is rarely seen (5).

Babesiosis was recorded in various domestic and wild animals in Iraq, with variation in proportions of infection depended upon many factors like age, breed, season and activity of ticks (6 and 7). Most of the previous studies depended in detection of parasite upon the microscopically examination, so the aim of this study was to detect the blood parasite (*B. bovis*) genetically in addition to show the effect of age and sex of animals on the infection rate.

Materials and Methods

This study was conducted during the period from December 2013 to August 2014 in different areas in addition to the abattoir of AL-Qadisiyah province. A total of 96 blood samples were collected from cattle clinically suspected to be infected with babesiosis. The examined cattle included 66 males and 30 females, where divided according to the age into three groups involved calves less than six months (≤ 6 m), young cattle ranged between six months to one year (6m – 1y)and adult cattle with age of more than one year (≥1y).

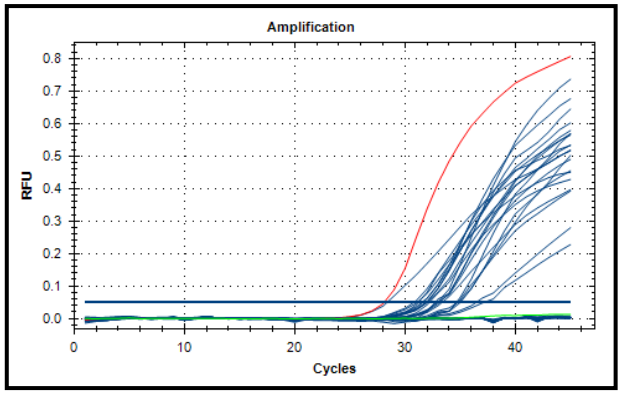
Two – five ml of blood sample were collected directly from the jugular vein or during the slaughtering and kept in anticoagulant EDTA tubes, then the samples were transferred in cooling conditions to the laboratory of Parasitology in Veterinary Medicine College in AL-Qadisiyah University.

DNA extractions from blood samples were done by used the Genomic DNA extraction Kit (Bioneer/Korea) according to the manufacturers instruction. The extracted DNA were tested by RT-PCR technique through used the RT-PCR kit (Genkam/Germany) for *B. bovis*, the thermocycler conditions was done according to primer annealing temperature and probe that included one cycle of pre-denaturation in 95 °C for 5 min, and 45 cycles of denaturation in 95 °C for 15 sec,

annealing/extension in 60 °C for 30 min and detection (scan) was 60 °C for 30 min.

Results and Discussion

The results showed that out of 96 babesiosis suspected cases were examined by real-time PCR. 47.91% (46/96) were infected with *B. bovis* (Fig. 1).



Figure, 1: Real-Time PCR amplification plot for *Babesia bovis* in positive and negative samples.

According to the age the highest infection (78.12%) were seen among cattle with age of more than 1 year (≥1y) with significant difference (P ≤0.05). (Table, 1).

Table, 1: Real time-PCR positive cases of *Babesia bovis* according to age.

Age	Examined No.	Positive No.	%
≤ 6 m	32	6	18.75 a
6 m-1y	32	15	46.87 b
≥ 1y	32	25	78.12 c
Total	96	46	47.91

Different letters refer to significant difference at (P ≤0.05).

Regarding to the sex the results appeared that the females cattle recorded the higher rate (50%) of infection when compared with the males, but with non-significant difference (P>0.05) (Table, 2).

Table, 2: Positive cases of *Babesia bovis* in Real time-PCR according to animal sex

Sex	Examined No.	Positive No.	%
Male	66	31	46.96 a
Female	30	15	50 a
Total	96	46	47.91

Similar letters refer to the non-significant difference at (P > 0.05).

In the current study and according to the RT-PCR technique, the result showed that 47.91% of suspected cases appeared positively to infection with *B. bovis*. Through access to the results of other studies, they found that most of them had the lowest results, than come in the current study, as indicated by Abdo-Sakaya (8) from Egypt, who said that the rate of infection with *Babesia* spp. reached 25.33% also scores of (9-11) ratios close of approximately 11%, also 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 (13-15). The difference among the current study and other studies may be attributing to difference in the samples number, climate conditions, spread of parasite and vector. Also the prevalence of infection and the occurrence of disease are determined by complex interactions, between the bovine host, vector and parasite (16). The present study pointed to that infection with babesiosis according to age revealed high percentage (78.12%) in cattle more than one year, whereas the lowest result were recorded in ages under six month (≤6m).The highest rate of infection in adult animals may be due to the chronicity of infection which can easily detectable by real time-PCR. Results above don't correspond with (17) who recorded rate of 28% in calves and 15% in adult cattle.

According to the sex the result appeared that non-significant differences between males and females in *B. bovis* infections. This result is in agreement with (18) and that may be due to both sexes subjected to the same conditions of rearing such as nutrition and climate.

Conclusion: The cattle in Al-Qadisiyah province are bearing the infection of *B. bovis* predominantly as a carrier hosts

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التحري الجزيئي لطفيلي *Babesia bovis* في الأبقار في محافظة القادسية

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الخلاصة

أنّ الهدف من هذه الدراسة هو تحديد الاصابة بطفيلي *Babesia Bovis* اعتماداً على الطريقة الوراثية. تم جمع 96 عينة دم من أبقار حية وأبقار مذبوحة من مناطق مختلفة بالإضافة الى مجزرة في محافظة القادسية، في المدة من شهر كانون الأول 2013 ولغاية شهر آب 2014. استعملت طريقة تفاعل السلسلة المتبلمرة في الوقت الحقيقي لتحديد وجود الطفيلي مع تأثير عمر وجنس الحيوان في نسبة الإصابة. أظهرت النتائج أنّ 47.91 % (96/46) من الأبقار المفحوصة كانت مصابة بطفيلي *B. bovis* وقد تركزت الإصابة أكثر في الأعمار الكبيرة (اكثر من سنة واحدة)، مع ملاحظة وجود اختلاف في نسب الإصابة بين الذكور والاناث ولكن بدون فرق معنوي ($P>0.05$). من ذلك نستنتج أنّ اغلب الأبقار في محافظة القادسية مصابة بطفيلي *B. bovis* بصورة دائميه، مما يجعلها كمضائف حاملة للإصابة.

الكلمات المفتاحية: داء الكمثريات ، *Babesia bovis* ، الابقار.