Molecular study of *Anaplasma marginale* parasite in carrier cattle in Al-Nasiriyah city

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Abstract

To detect *Anaplasma marginale* among carrier cattle by using polymerase chain reaction (PCR) technique, 64 blood samples, from healthy cows in abattoir of Al-Nasiriyah city were collected from June till August, 2017 in this study. By targeting MAR1bB2 gene with the molecular weight of approximately 265 bp, *Anaplasma marginale* were detected in18 samples (28.125%). One of these positive sample was recoded in National Center for Biotechnology Information, NCBI; Gene Bank.

Keywords: Anaplasmosis, *Anaplasma marginale***,** Protozoa, PCR
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دراسة جزئية لطفيلي *Anaplasma marginale* في الماشية الحاملة للاصابة في مدينة الناصرية

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الخلاصة

غرض التحري عن *Anaplasma marginale* في الماشية الحاملة للاصابة باستخدام تقنية تفاعل البوليميريز المتسلسل، جمع 64 نموذج دم من ابقار سليمة مظهريا في مجزرة الناصرية للفترة من حزيران الى اب 2017 في هذه الدراسة. عن طريق استهداف الجين MAR1bB2 بوزن جزيئي يقارب 265 bp، اكتشفت *Anaplasma marginale* في 18 نموذجا (28,125%) وسجل احد النماذج الموجبة في المركز الوطني لمعلومات التقانات الحيوية، بنك الجينات.

Introduction

Anaplasma marginale,the tick-borne obligate intraerythrocytic pathogens cause bovine anaplasmosis in cattle and wild ruminants (1). In addition to biological transmission by ixodid ticks, Mecanichal transmission of bovine anaplasmosis may also be occurred by biting of flies or blood contaminated tools (2). The study of (3) reported that *Anaplasma marginale* can be transmitted transplacentalLy. A high economic losses due to bovine anaplasmosis were reported in the tropical and subtropical area, as well as, this disease cause a huge problems for management and health of livestock in these regions (4). Cattle which were recovered clinically from this disease may become carrier lifelong and serve as reservoir (5). In pre symptomatic and carrier animals,traditional diagnosis which based on microscopic examination of Giemsa stained blood smear may be useless (6), so this study aimed to investigate bovine anaplasmosis in carrier cattle in Al-*Nasiriyah* city by using polymerase chain reaction (PCR) technique.

Material and methods

Samples

To collect blood samples, 64 healthy cows from abattoir of Al-Nasiriyah city southern Iraq which were randomly selected from June till August, 2017. Blood samples were taken from jugular veins of these cows. By using ice-pack containers these blood samples were brought

to PCR unit in college of veterinary medicine, university of Thiqar.

Extraction of DNA

A volume of 200 µl fresh blood was extracted by DNA extraction kit (geneaid) as recommended by Manufacturer.

PCR assay

PCR assay targeting MAR1bB2 gene an element of *A.marginale* (7) was used for detecting *A.marginale*. The primers (Forward: 5-GCT CTA GCA GGT TAT GCG TC-3/Reverse 50-CTG CTT GGG AGAATG CAC CT-3) were used to amplify a 265bp.

The PCR mixture (50 µl): DNA templates 10 µl, Mastermix 10 µl, Primer forward 2µl, Primer reverse 2 µl, DW 26 µl.

The PCR conditions for *A.marginale* included 1 cycle of an initial denaturation of DNA at 94°c for for 3 min. followed by 30 cycles of denaturation (95c for 50 s), primer annealing (50c for 50 s) and extension at 65°c for 1 min. A final extension at 65°c for 10 min was performed. For each reaction, 10 µl of PCR product was electrophoresed on 2% agarose gel containing 1µl /ml ethidium bromide at 70 Voltage and visualized under U.V. light.

DNA sequencing

Only two PCR products of MAR1bB2gene in *A.marginale* isolates were chosen for sequencing, and forward primer for this gene, and sent outside Iraq to be sequenced (Macrogen, Korea). Basic Local Alignment Search Tool analysis (BLAST) was lead to blast algorithm. The samples sequences were edited, aligned, and compared with the reference sequences using sequence alignment (8).

Results and discussion

The previous studies indicate that msp1b of *A. marginale* genome is a very sensitive and specific target for detection of *A. marginale* infection in ticks and in cattle hosts (6,9). The primer set successfully amplified all available stocks and the sequence of PCR amplified fragments also sensitivity and specificity of this primer was shown to be identical at the nucleotide sequences. These results suggest that the MAR1bB2 primers may be suitable to detect *A. marginale* isolates from world. In this study, out of 64 collected blood sample, only18 (28.125%) was identified as *Anaplasma marginale* which amplified the targeted gene, with the molecular weight of approximately 265 bp (Fig. 1).

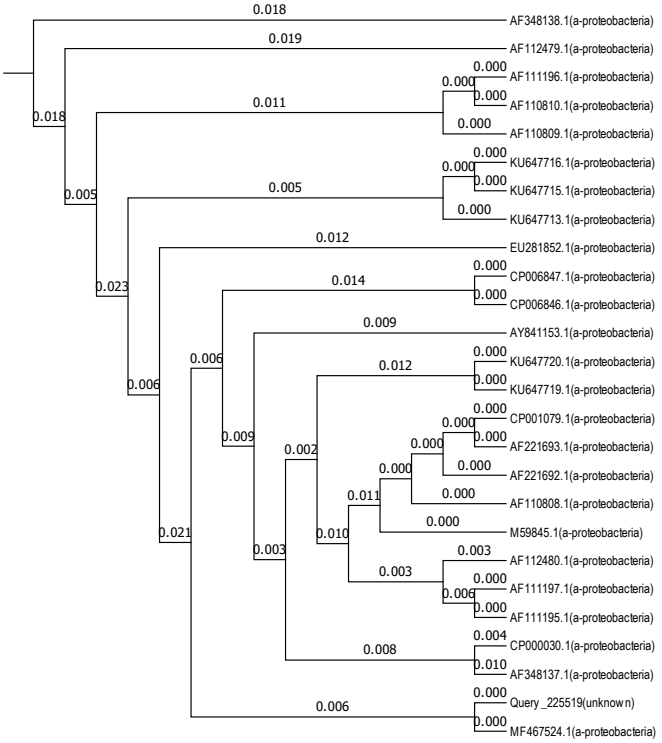

Figure 1: Evolutionary relationships of taxa, based on *MAR1bB2*gene gene partial sequence that used for *Anaplasma marginale*detection from animals samples.

One of these possitive samples was recoded in National Center for Biotechnology Information, NCBI; Gene Bank, under name (*Anaplasma marginale* strain abbas4 major surface protein 1b MAR1bB2 gene, partial cds). under accession number MF467524. Evolutionary analyses were conducted in MEGA7 (4). *A. marginale* infection was reported by many studies done in different parts of Iraq. The result of current study is higher than infection rate reported by (10) who recorded 9.09% of cattle in Erbil and (11) who reported in 13.04 % of cattle in Wassit by using ELISA test. The infection rate of this study is lower than73% of cattle by competitive ELISA which published by (12) in Al-Nasiriyah city, 30.4% of cattle which recorded by (13) in Al-Diwanyia and 35% of cattle in Wassit which recorded by (14) by using ELISA test. These differences of the results may be due to alternative diagnostic techniques and different environmental conditions of these different area.

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