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Research article

Tick-borne pathogens: *Anaplasma* spp as an example

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Abstract

With the aim of study tick-borne pathogens and identify some of these microorganisms in the tissues of ticks that affect cows in Al-Diwaniyah City, Iraq, we focused on detecting Anaplasma spp as one of these pathogens that are transmitted by these vectors. Here, we collected 150 ticks from 200 affected cows from the mentioned city. The ticks were first identified for morphological-based naming of the genus that they belonged to by sending samples to the Iraqi Natural History Museum, Baghdad, Iraq. Then, the extracted DNA from the ticks was subjected to partial DNA-based sequencing of Anaplasma spp. The results of the morphological study identified the ticks from Hyalomma spp. the sequencing resulted in detecting and confirming the presence of Anaplasma spp in the tissues of the ticks in which 2 isolates were provided. The phylogenetic-based investigation provides 2 isolates of Anaplasma spp, SP1 (MH119129.1) and SP2 (MH119130.1). These isolates were branched up in the phylogenetic tree close to global isolates, KT264188.1, from Thailand. These results give interesting and important information about the dangerousness of ticks via their transmission of these pathogens to healthy animals such as Anaplasma spp.

Keywords: *Anaplasma*, phylogeny, ticks**.**

Introduction

Ticks act in very important and dangerous role in transmitting various types of pathogens such as *Babesia*, *Theleiria*, and *Anaplasma*. According to many studies and observations that previously were performed, *Anaplasma* spp is considered as a major intruder that ticks transmit to animals. The disease is well-known for the huge economic setbacks that it induces to industries or to the health of animals (2, 4). To stand on the reason for this problem, findings provide generous information that these tick-borne pathogens initiate high level of animal death due to these diseases (2, 8, 9, 14). Deeper look at these reasons, infectiveness of this pathogen to red blood cells (RBCs) increases the rate of intravascular destruction of these cells in macrophages leading to various degrees of anemia that could reach to the severe level (14). The long time, up to 60 days, between infection and appearance of a clinical form of *Anaplasma marginale* ends up with fever, signs of anemia such as paleness, relevant anorexia, obvious lethargy, and noticed icterus (3, 7). Liver- and spleen-destruction-based anemia and subsequent decreased O_2 level in vital organs lead to a bad prognosis that may lead to the death of the affected animal (7). In the current investigational-based work, we intended to study the pathogens, *Anaplasm* spp as an example, which are transmitted by the important vector, ticks, plus study the evolution history of these microorganisms in Al-Diwaniyah City, Iraq.

Materials and Methods

Ethical approval

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The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study.

Sampling

We collected 150 ticks from 200 affected cows from Al-Diwaniyah City, Iraq. The ticks were first identified for morphological-based naming of the genus that they belonged to by sending samples to the Iraqi Natural History Museum, Baghdad, Iraq and using (6) as a reference for key features of morphology.

DNA extraction

DNA regarding the tick tissues and the pathogens was extracted using gSYAN DNA mini extraction kit (Geneaid, USA). Here, homogenization process was done using crushed tick-based tissues, samples of 200mg, which were place in tubes. Then, the process of DNA extraction was generated following the protocol provided by with the kit. After that, quantification and qualification processes of the produced DNA were followed using a NanoDrop.

Polymerase chain reaction

Depending on the NCBI-based designed primer; F: ATGAGTGCTGAATGTGGGGG and R: GCAGTGTGTACAAGACCCGA, which was ordered from (Bioneer Company, South Korea), 584-bp-targeted region in the *16S rRNA* gene was employed to be amplified in a PCR-based technique. The kit, AccuPower® PCR PreMix kit (Bioneer Comp. South Korea), was utilized to generate the matermix. Following the instructions provided with the kit, the mix contained DNA polymerase 1U, dNTPs 250 µM, Tris-HCl (pH 9.0) 10 mM, KCl 30 mM, $MgCl_2$ 1.5 mM, stabilizer, tracking dye, 5µl DNA, 1.5µl of 10pmole of each primer, and 12µl PCR water. The thermocycler conditions were primary denaturation 95℃ for 5min, 30 cycles of (principle denaturation 95°C for 1min, the process of annealing 58°C for 1min, and a process of extension 72°C for 1min), and finishing extension at 72°C for 10min. The process regarding 1.5%-agarose-gel-based electrophoresis was initiated incorporating with ethidium bromide use. The final results were collected after UV-based visualizing of the gel products was made.

Partial DNA sequencing

The PCR-based amplified products were sent out for sequencing. NCBI- and MEGA 6.0-based processing was utilized to recognize the isolates, their matching to other isolates of the world, and build up the phylogenetic tree following the evolutionary distances via Maximum Composite Likelihood Method (12, 13).

Results

Tick morphology

According to the results provided by The Iraqi Natural History Museum, genus *Hyalomma* spp was the most identified genus in this study.

Phylogeny

The sequencing resulted in detecting and confirming the presence of *Anaplasma* spp in the tissues of the ticks in which 2 isolates were provided. The phylogenetic-based investigation provides 2 isolates of *Anaplasma* spp, SP1 (MH119129.1) and SP2 (MH119130.1). These isolates were branched up in the phylogenetic tree close to global isolates, KT264188.1, from Thailand as shown in figure (1).

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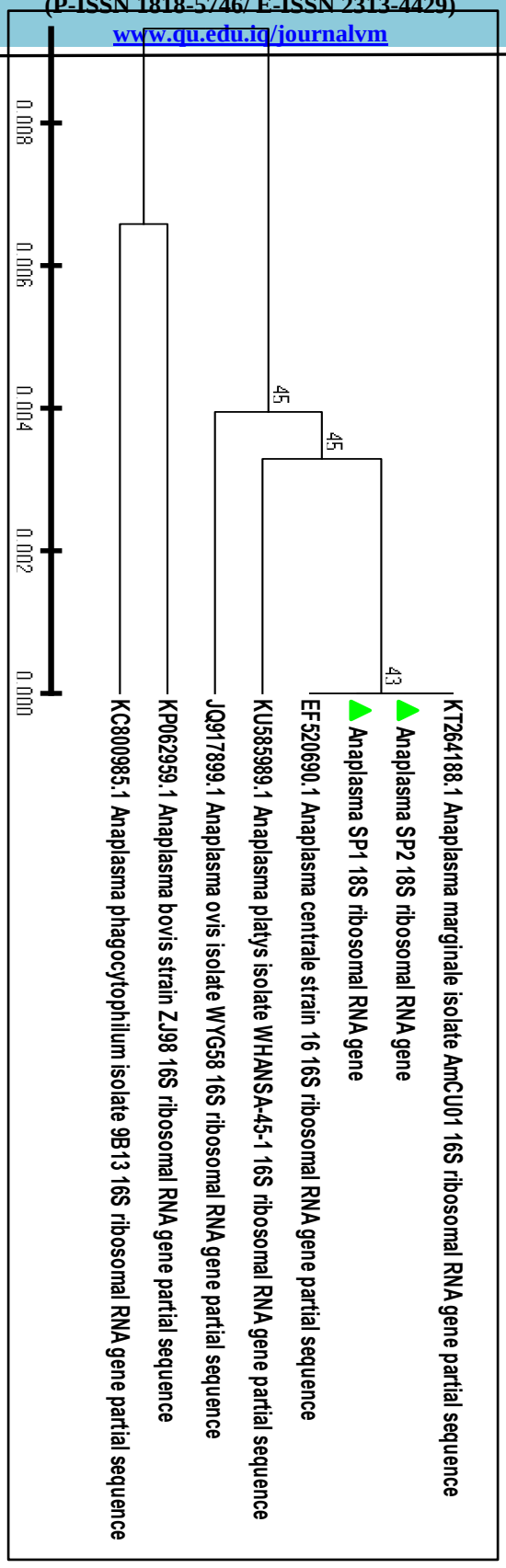

Figure (1): Tree of phylogeny. *Anaplasma* current isolates (SP1 and SP2)

Discussion

Ticks act in very important and dangerous role in transmitting various types of pathogens such as *Babesia*, *Theleiria*, and *Anaplasma*. According to many studies and

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observations that previously were performed, *Anaplasma* spp is considered as a major intruder that ticks transmit to animals. The disease is well-known for the huge economic setbacks that it induces to industries or to the health of animals (4). The results of the PCR indicated that these pathogens were *Anaplasma* spp. The importance of the recorded results is that it gives reliable data about the presence of the disease in tested city. When researchers need reliable methods to detect this pathogen, this result identify PCR as a good technique to follow for trusted diagnosis (1, 5, 10) who provide 92%-based reliability results of using PCR. Our results agree with (11) who detected this microorganism in small ruminants, sheep. Our isolates, according to partial sequencing, were placed via the phylogenetic tree to match with certain global isolates such as the one from Thailand. These results provide valued data to be used for better understanding of the tick-borne pathogens in the city of Al-Diwaniyah, Iraq.

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