Sequencing-based phylogenetic-study of *Babesia* spp detected in tick tissues in Al-Diwaniyah province, Iraq

M.S. Hajeel and M.A.A. Al-Fatlawi*

Department of Microbiology, College of Veterinary Medicine, University of Al-Qadisiyah, Al-Diwaniyah, Iraq
* email: monyerr.abd@qu.edu.iq

(Received April 8, 2018; Accepted May 21, 2018)

Abstract

Our study purpose was to investigate the evolution of *Babesia* spp isolated from tissues of ticks that were found on 150 cows in Al-Diwaniyah province, Iraq. To fulfill the required purpose, sampling of 10 ticks was performed from each infested cow. These obtained ticks were morphologically recognized first, and then they were introduced to Lab investigation that was started with crushing the tick tissues to extract the genomic DNA of the *Babesia* spp. The DNA was then applied to polymerase chain reaction (PCR) method to recognize the amplification of the region that is related to the 18S rRNA gene. The resulted-amplified products were sequenced for the purpose of confirming and doing the phylogenetic analyses. Here, our study has demonstrated 2 different species according to the results of the sequencing and the phylogenetic analyses of the tested *Babesisa* species. These 2 species are SP1 and SP2. When the phylogenetic tree was built up, the results showed that SP1 and SP2 are closely related to *Babesia bovis* (HQ264126.1), an isolate from Texas, USA. Our study indicates interesting and valued data that could be used to study various aspects of the tick, *Babesia* species, and their control in Al-Diwaniyah City, Iraq.

Keywords: PCR, phylogeny, *Babesia*, tick
Available online at http://www.vetmedmosul.com

دراسة تعاقبية للقواعد النتروجينية ونسلية للـ *Babesia* spp المحددة في انسجة القراد في مدينة الديوانية، العراق

مروة سليم هجيل و منير عبد الأمير عبد الفتلاوي

فرع الأحياء المجهرية، كلية الطب البيطري، جامعة القادسية، الديوانية، العراق

الخلاصة

هدفت دراستنا التحري عن تطور الـ *Babesia* spp المعزولة من أنسجة القراد الذي وجد على ١٥٠ بقرة في مدينة الديوانية، العراق. لغرض تحقيق ذلك تم اخذ ١٠ قرادات من كل بقرة مصابة. تم تمييز هذه القرادات مظهريا في بادئ الأمر، وبعد ذلك تم معاملة هذه القرادات مختبريا والتي بدأت بسحق أنسجة تلك القرادات لاستخلاص الـ DNA للـ *Babesia* spp. عرض الـ DNA بعد ذلك لطريقة تفاعل أنزيم البلمرة المتسلسل (PCR) لتميز تضخيم المنطقة العائدة للجين 18S rRNA. درس المنتوج المضخم تعاقبيا بالنسبة للقواعد النتروجينية لتاكيد التشخيص وعمل التحليلات النسلية. هنا، دراستنا عرضت نوعين اعتمادا على النتائج الخاصة بالتعاقب والتحليلات النسلية لانواع *Babesia* المفحوصة. هذه الانواع هي SP1 و SP2. عندما بنيت الشجرة النسلية، أظهرت النتائج أن SP1 و SP2 قريبة جدا من *Babesia bovis* (HQ264126.1)، عزلة من ولاية تكساس، الولايات المتحدة الأمريكية. تشير دراستنا إلى بيانات قيمة ومثيرة للاهتمام والتي ممكن استخدامها لدراسة جوانب مختلفة من القرادة، أنواع الـ *Babesia*، والسيطرة عليهما في مدينة الديوانية، العراق.

Introduction

The diseases that are caused by protozoa in animals induce major crises of economic and health losses around the world (1). *Babesia*-species members are globally recognized for their huge damages that they cause to the world industries of herds (2). Babesiosis is a tick-borne disease that generates a blood-based disease in different animals such as cows (3). Different genera and species of ticks such as *Hyalomma* and *Rhipicephalus* are well-known for their enhancement to transmit *Babesia* species to different animals (4). This microorganism has a lifestyle of asexual proliferation in animal hosts and a sexual-based life-style in ticks (5). A regular, but not too much trustworthy, diagnostic tool is the microscopic diagnosis of *Babesia* spp that has been used for decades till now to perform the diagnosis of this protozoan. However; relying on a high-error tool may lead to obtain false-negative results especially when having low numbers of parasites in blood samples of the tested animals that could be happened in subclinical and carrier cases. Microscopic diagnosis, sometimes, misses the capabilities to recognize between species level leaving the door open for indefinite diagnosis (6). For better diagnosis and identification of *Babesia* spp, molecular-based techniques such as PCR and sequencing of a specific-piece of sequence of a specific gene such as 18S rRNA gene are needed to promote the processes of correct characterization, identification, and diagnosis of *Babesia* species that affect animals. These techniques are specially required when having low numbers of parasites in blood specimens of the studied animals (7). True insight information about the presence of certain species of *Babesia* in a given area of the world is generated via the use of molecular-based procedures such as sequencing and phylogenetic analyses (8). PCR and partial-18S-rRNA-gene sequencing were recruited to molecularly analyze *Babesia* spp isolated from ticks that affected cows in Al-Diwaniyah province, Iraq.

Material and methods

Sampling and DNA extraction

Our study purpose was to investigate the evolution of *Babesia* spp in tissues of ticks that were found on 150 out of 200 examined cows in Al-Diwaniyah province, Iraq. To fulfill the required purpose, sampling of 10 ticks was performed from each infested cow. These obtained ticks were morphologically recognized first according to (9) in the Iraqi Natural History Museum, and then they were introduced to lab investigation that was started with crushing 150 ticks to extract the genomic DNA of the *Babesia* spp. Extraction of the DNA was performed via the employment of gSYAN DNA mini extraction kit (Geneaid, USA), a tissue protocol. To prepare the tick tissues for the extraction process, an amount of 200 mg of tick tissues was added in a 1.5-ml tube, and an amount of 200µl of GST puffer was inserted into the tube. Then, crushing and homogenization steps were performed utilizing a micropestle. A NanoDrop was recruited to measure the DNA quality and quantity.

Polymerase chain reaction and gel electrophoresis

For the preparation of the mastermix, AccuPower PCR PreMix Kit was employed using DNA 5 µl, 10 pmol at 1.5 µl of FP: GGGACGCCTCGTTACTTTGA and 10 pmol at 1.5 µl of RP: TCCACCAACTAAGAACGGCC, 12 µl H_2O for molecular biology. The kit protocol was used to finish the preparation process. NCBI website and primer 3 plus (Macrogen Company, Korea) were utilized to pick up the right primers. The PCR product at 551 bp of the 18S rRNA gene was targeted by the PCR amplification step. The conditions of the reactions were as follows: 1-cycle-94 ℃-5min of initial denaturation, 35 cycles of 94 ℃-30 sec denaturation, 58 ℃-30 sec annealing, and 72 ℃-1 min extension, and 1-cycle-72 ℃-5 min of final extension. Bands of the PCR products were transferred for separation purposes using electrophoresis via the use of 1.5% agarose gel at 100 volt and 80 amp for duration of 1hour. A UV imager was employed to visualize these product bands.

18s rRNA gene partial sequencing

Five-positive products at 551 bp regarding the 18S rRNA gene were sequenced. The sequences were NCBI-based processed to identify our obtained isolates using known world species. The phylogenetic tree was built up employing MEGA 6.0 software according to the basis of evolutionary distances via Maximum Composite Likelihood Method (10,11).

Results

The resulted-amplified products were sequenced for the purpose of confirming and doing the phylogenetic analyses. Here, our study has demonstrated 2 different species according to the results of the sequencing and the phylogenetic analyses of the tested *Babesisa* species. These 2 species are SP1 (MH109518.1) and SP2 (MH109519.1). When the phylogenetic tree was built up, the results showed that SP1 and SP2 are closely related to *Babesia bovis* (HQ264126.1), an isolate from Texas, USA, Figure 1. Our study indicates interesting and valued data that could be used to study various aspects of the tick, *Babesia* species, and their control in Al-Diwaniyah province, Iraq.

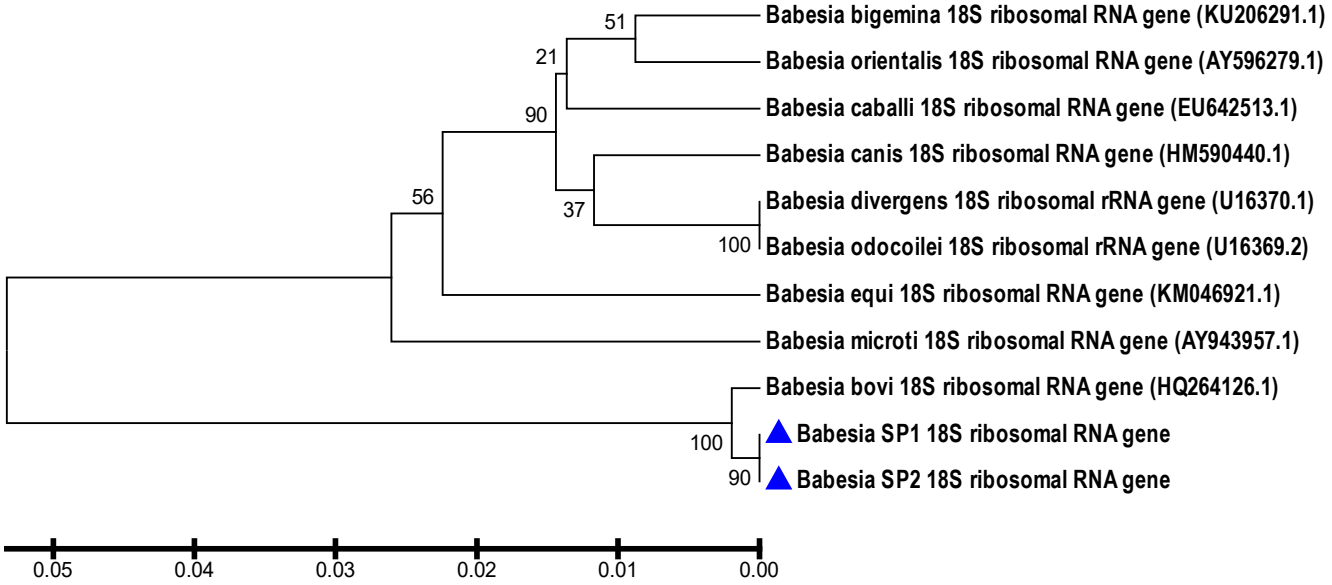

Figure 1: Phylogenetic tree of *Babesia* spp via the use of partial sequence of 18S rRNA gene, SP1 and SP2 are the current study species.

Discussion

The diseases that are caused by *Babesia* spp are diseases for global importance due to their effects on the economy and health system regarding food animals in the countries that these diseases are considered part of their listed tick-borne diseases (12). The current study has utilized the 18S-rRNA-gene-based sequencing to molecularly investigate the reality of species known to cause diseases in cows in Al-Diwaniyah City, Iraq. This sequencing has generated appealing findings regarding 2 isolated species of *Babesia* spp that were stand together in a very close neighboring to an isolate, *Babesia bovis* (HQ264126.1), from Texas, USA. The HQ264126.1 was identified using the partial sequencing of 18S rRNA gene of *Babesia bovis* from ticks infested white-tailed deer (13). This indicates that our species and the Texas one might have descended from one ancestor, and there are factors that play roles in spreading this ancestor around the world such as moving of animals and people and importation/exportation processes. This could also indicate that these isolates might have descended from a specific species related to different parts of the world other than USA or Iraq. The present-study findings agree with (14,15) that identified *Babesia* spp via the use of molecular-based tools to diagnose and characterize the microorganism in the tested animals. Our study indicates interesting and valued data that could be used to study various aspects of the tick, *Babesia* species, and their control in Al-Diwaniyah City, Iraq. The big effect of ticks in transmitting *Babesia* species between cows is also unveiled by the information provided by the current study results. The accurate control of the diseases that are caused by *Babesia* spp needs to take in consideration important factors such as cost, validity, and the urgency in taking actions when an outbreak occurs. To fulfill these three factors, insight learning of the life of the protozoa in the city should be followed using various molecular methods.

References

1. Qiu Y, Kaneko C, Kajihara M, Ngonda S, Simulundu E, Muleya W, Thu MJ, Hang'ombe MB, Katakura K, Takada A, Sawa H, Simuunza M, Nakao R. Tick-borne haemoparasites and Anaplasmataceae in domestic dogs in Zambia. Ticks Tick Borne Dis. 2018;doi:10.1016/j.ttbdis.2018.03.025.
2. Greay TL, Zahedi A, Krige AS, Owens JM, Rees RL, Ryan UM, Oskam CK, Irwin PJ. Endemic, exotic and novel apicomplexan parasites detected during a national study of ticks from companion animals in Australia. Parasit Vectors. 2018;11(1):197. doi:10.1186/s13071-018-2775-y.
3. Da Rold G, Ravagnan S, Soppelsa F, Porcellato E, Soppelsa M, Obber F, Citterio CV, Carlin S, Danesi P, Montarsi F, Capelli G. Ticks are more suitable than red foxes for monitoring zoonotic tick-borne pathogens in northeastern Italy. Parasit Vectors. 2018;11(1):137. doi:10.1186/s13071-018-2726-7.
4. Rajabi S, Esmaeilnejad B, Tavassoli M. A molecular study on Babesia spp. in cattle and ticks in West-Azerbaijan province, Iran. Vet Res forum Int Q J. 2017;8(4):299-306.
5. Hussein HE, Bastos RG, Schneider DA, et al. The Babesia bovis hap2 gene is not required for blood stage replication, but expressed upon in vitro sexual stage induction. PLoS Negl Trop Dis. 2017;11(10):e0005965.
6. Hailemariam Z, Krücken J, Baumann M, Ahmed JS, Clausen P-H, Nijhof AM. Molecular detection of tick-borne pathogens in cattle

from Southwestern Ethiopia. PLoS One. 2017;12(11):e0188248. doi:10.1371/journal.pone.0188248

7. Criado-Fornelio A. A review of nucleic-acid-based diagnostic tests for Babesia and Theileria, with emphasis on bovine piroplasms. Parassitologia. 2007;49(Suppl 1):39-44.
8. Criado-Fornelio A, Martinez-Marcos A, Buling-Saraña A, Barba-Carretero J. Molecular studies on Babesia, Theileria and Hepatozoon in southern Europe: Part II. Phylogenetic analysis and evolutionary history. Vet Parasitol. 2003;114(3):173-194. doi:10.1016/S0304-4017(03)00141-9.
9. Shubber H, Mohammed M, AlHassani N. Taxanomic, anatomic and molecular study of ixodid ticks parasitizing some mammals and birds in the middle and south of Iraq. PhD Thesis, University of Al-Qadisiyah. 2014.
10. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987; 4:406-25. http://www.ncbi.nlm.nih.gov/pubmed/3447015 (accessed December 16, 2017).
11. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0., Mol Biol Evol. 2013;30:2725-9. doi:10.1093/molbev/mst197.
12. El-Ashker M, Hotzel H, Gwida M, El-Beskawy M, Silaghi C, Tomaso H. Molecular biological identification of Babesia, Theileria, and Anaplasma species in cattle in Egypt using PCR assays, gene sequence analysis and a novel DNA microarray. Vet Parasitol. 2015;207:329-334. doi:10.1016/J.VETPAR.2014.12.025.
13. Holman PJ, Carroll JE, Pugh R, Davis DS. Molecular detection of Babesia bovis and Babesia bigemina in white-tailed deer (Odocoileus virginianus) from Tom Green County in central Texas. Vet Parasitol. 2011;177(3-4):298-304. doi:10.1016/j.vetpar.2010.11.052.
14. Elbaz E, Moustafa MAM, Lee K, Mohamed WMA, Nakao R, Shimozuru M, Sashika M, Younis EEA, El-khodery SA, Tsubota T. Molecular identification and characterization of piroplasm species in Hokkaido sika deer (Cervus nippon yesoensis), Japan. Tick Borne Dis. 2017;8:802-807. doi:10.1016/j.ttbdis.2017.06.007.
15. Jirapattharasate C, Adjou Moumouni PF, Cao S, Iguchi A, Liu M, Wang G, Zhou M, Vudriko P, Efstratiou A, Changbunjong T, Sungpradit S, Ratanakorn P, Moonarmart W, Sedwisai P, Weluwanarak T, Wongsawang W, Suzuki H, Xuan X. Molecular detection and genetic diversity of bovine Babesia spp., Theileria orientalis, and Anaplasma marginale in beef cattle in Thailand. Parasitol Res. 2017;116:751–762. doi:10.1007/s00436-016-5345-2.