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Molecular Detection of *Babesia bovis* Isolated from Cows in Thi-Qar Province

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Abstract-The present study was designed to determine the *bovar2A* gene in *Babesia bovis* Thus gene encodes a variant erythrocyte surface antigen that is unique to *B. bovis* was detected in the blood of infected cows in Thi-Qar province during the period of February to June 2019. A total of 96 blood samples were collected from the cows of both sexes. The Polymerase Chain Reaction (PCR)is used to detect the *bovar2A* gene in Babesial infection status in cows. The PCR was used to distinguish *Babesia bovis* with a high level of sensitivity, capable to detection every species of *B. bovis*, and no evidence of non-specific amplification. The PCR results showed the *bovar2A* gene was harbored in 64 of 96 samples (66.66%) of *Babesia bovis*. The current study concluded that the *bovar2A* gene is useful for diagnosing *B. bovis* and the rising incidence of the disease in southern Iraq.

Keywords: *bovar2A gene, Babesia bovis, PCR*

I. Introduction

In tropical and subtropical regions, B. bigemina and B. bovis are the primary vectors of the tick-borne illness bovine babesiosis. There have been reports that Babesia bovis-caused babesiosis is more severe than Babesia bigemina-caused babesiosis [1,2]. Low numbers of erythrocytes remain infected with Babesia bovis 2 in a long-lasting carrier status that develops after an animal recovers from infection. Despite being a common method for diagnosing babesia infections, the detection of parasites in blood smears has shown poor sensitivity throughout the subclinical and chronic stages of the illness. Higher sensitivity and specificity were demonstrated by molecular tools. [3,4]

In Iraq, babesiosis was found in a variety of domestic and wild animals, with the percentage of infection varying depending on age, breed, season, and tick activity [5,6]. More sophisticated and sensitive methods, like nucleic acid-base
detection techniques, should be employed in preclinical and chronic infections to detect parasites with low parasitemia levels [7]. Furthermore, the detection of numerous hemoprotozoa in a single reaction will be more cost-effective and efficient with multiplex PCR [8]. Babesia bovis was to be detected by molecular technique utilizing PCR because the majority of earlier investigations relied on the detection of parasites in microscopic inspection. The present study aimed to detect the *bovar2A* gene used for the diagnosis of B. bovis in the blood of infected cows by the PCR technique.

II. Material and Methods

Ninety-six blood samples were collected from infected cows suffering from enlargement of lymph nodes and eye paleness, High fever, Anorexia, Lethargy, weakness and reluctance to move; from the jugular vein in tubes with anticoagulant (EDTA). The cows were referred to a particular veterinary clinic in the Thi-Qar government.

Extraction of the DNA from the blood samples was done with a commercial kit using the Genomic DNA Extraction kit (Geneaid/Korea). The PCR mixture was achieved with a final volume of 20ul, and the deionized free water used as a negative control. The detection of B. bovis was performed by amplifying *bovar2A* gene by using two primers as the following: primer 1 forword: (5'-CCCTGACCAGCACAAACGA -3') 192 bp and reverse' 5' -TTACAATGCGCCAGCAGTCC -3') and primer 2 forword 5' - GAAAGAGGCGGACCATGAGA-3' and reverse 5' -TGTGCTGGTCAGGGTCACT-3' 845bp under the following protocol: 94°C for 3min in the initial denaturation, (95°C for 50 sec, 55 to primer 1 and 54°C to primer 2 for 50sec, 65°Cfor 1 min) for 35 cycles and 65°C for 10 min for the final extension7.

III. Results and Discussion

For both humans and animals, babesiosis was a serious and invasive disease. The biblical Book of Exodus describes cow mortality as the first instance of an epidemic caused by the Babesia genus. Subclinical babesiosis causes cattle to become chronic piroplasm carriers, which makes them reservoirs of infection for tick vectors. Latent infections, however, were crucial to the epidemiology of this illness.

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The existing study recorded that 64/96 (66.66%) of *B. bovis* harbored the goal gene and recorded as positive results, as shown in Figure (1)and Figure (2)of primer 1 and 2, respectively.

The bovarZA primer was specific to detect B. bovis, also Ferreri *et al.*, whom suggest the biovar2A primer might be used to identify the B. bovis in diverse geographic provinces where the goal disease was endemic. The PCR technique was one of almost frequently used tools in recognition of Babesia. Therefore, the choice of suitable primers to identify the Babesia was tremendously imperative.

The present results agreed with the results of Sabbar and Aaiz[9]. whom showed a positive infection with Babesiosis. Among them, 54.92 % (57/97) had a positive result for B. bovis .

When target sequences are missing or the primer is inaccessible, PCR can produce negative results. The recent study documented that the occurrence of Babesia spp. was higher than that found in Thailand and China. In Argentina an occurrence of Babesia bovis ranging from 34 - 61 % was observed 9. The high frequency of babesiosis indicates a situation of stable endemicity [10]. Also, the recognition of babesiosis in the early stage of infection in both animals and in carriers by PCR was a powerful device for epidemiology, meanwhile those animals represent a chief cause of alimentary infections [11]. The serological diagnosis of babesiosis was slightly, so the serological test used to identify babesiosis was the immunoforescent assay, and seropositivity rates was 33.5% 1L12[12]. In Iraq, the prevalence of babesiosis was 27.2 by ELISA [113], and 38.54 by PCR [14].

Therefore, El-Naga and Barghash [15]. Tests of the two methods for detecting Babesia revealed that PCR had a greater detection rate (18.43%) than Giemsa staining (11.8%).

A single B. bovis infection was far more common in our study (21.21%) than a single B. bigemina infection (5.55%) [16]. This finding was in contrast to that of Oliveira et al. [17,18] and Adham et al. [19,20]. who discovered that in all cattle groups, cattle with mixed infection by multiple species—that is, cattle infected with both B. bovis and B. bigemina concurrently—tended to have a higher positivity percentage.

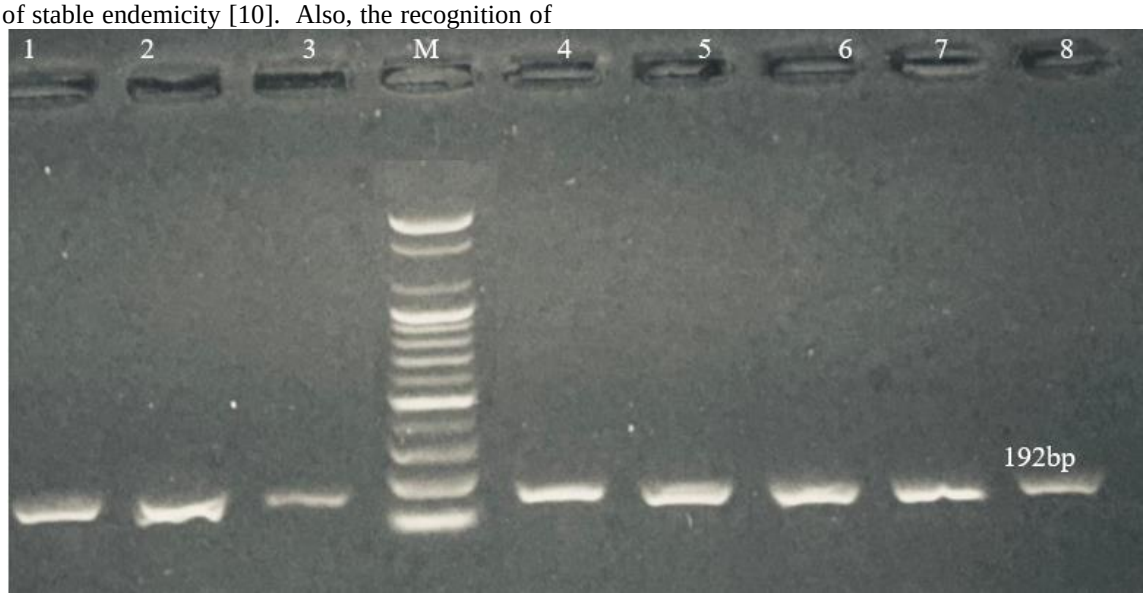

Figure (1): PCR amplification of a *bovar2A* gene192bp, line M (100 bp ladder); Lines 1-8 as positive results

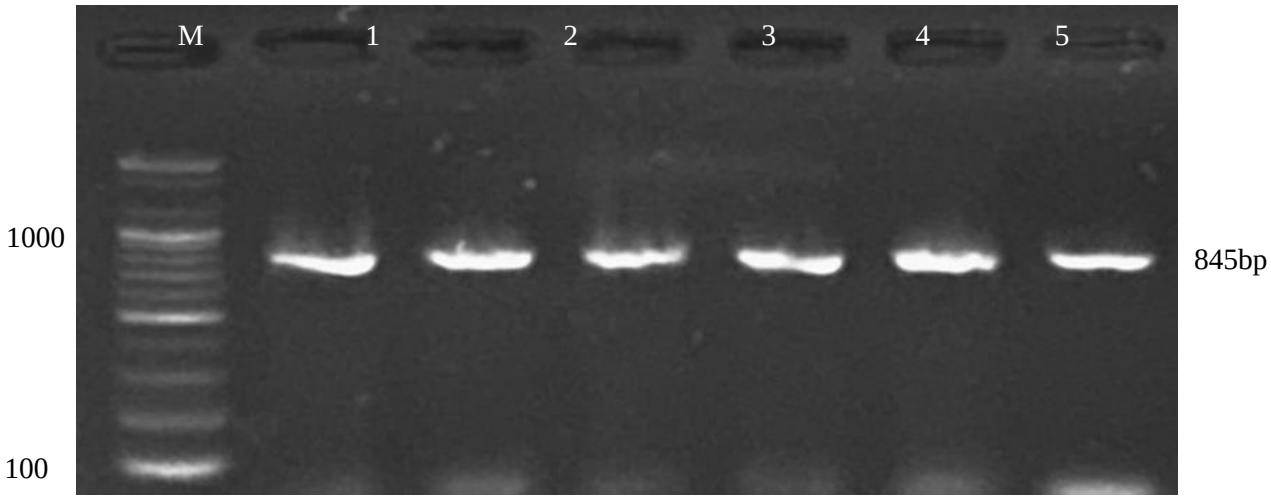

Figure (2): PCR amplification of a *bovar2A* gene, 845bp, line M (100 bp ladder); Lines 1-6 as positive results.

IV. Conclusion

The current study's findings demonstrated the importance of the bovar2A gene in diagnosing Babesia bovis and the rising incidence of the disease in southern Iraq.

The present study recommends performing a scopy of the parasite to identify genetic variations, and investigating the quality of meat infected with the parasite.

Conflicts of interest

The authors declare that they have no conflict of interest.

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