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# Prevalence of *Babesia bovis* and *B. bigemina* in animals slaughtered in Abha and Khamis Mushait abattoirs, Aseer, Saudi Arabia, using PCR assay

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**ABSTRACT**

*Babesia* spp. are malaria-like protozoans that parasitize and reproduce within mammalian red blood cells. These parasites have medical, veterinary and economic importance, since they caused significant losses in livestock and serious health problems to human. In spite of the great importance of these parasites, little information is available about the prevalence of them in the Kingdom of Saudi Arabia (KSA) generally and in Aseer region particularly. 350 blood samples were collected from 30 camels, 4 cows, 136 goats and 180 sheep slaughtered in Abha and Khamis Mushait abattoirs, Aseer region, KSA. Blood samples were transported to the lab and DNA was extracted from them. Such DNA was subjected to PCR assay using specific primers for *B. bovis* and *B. bigemina*. The results revealed that prevalence of *B. bovis* was 6.25, 0, 0.74 and 1.2 % for camels, cows, goats and sheep, respectively, however, all samples appeared negative to *B. bigemina.* This is the first report of *B. bovis* in Saudi Arabia. These results provided important information about Babesiosis prevalence in Southwestern region of KSA; in addition, the sensitive and accurate method of diagnosis used in this study will be helpful in large-scale monitoring of transmission foci in endemic areas and will be important for the long-term control of the disease.

**Key words:** *Babesia* spp., Saudi Arabia, Molecular Diagnosis, Prevalence

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## INTRODUCTION

Babesiosis is a worldwide tick-borne hemoprotozoosis affecting many mammalian species and caused by intraerythrocytic multiplication of apicomplexans in the *Babesia* genus. The evolutionary success of this parasite is attested by more than 100 species described [1]. It has been estimated that tick-borne diseases cause US $ 13.9 to 18.7 billion loss per annum and world's 80% cattle population are at risk of ticks and tick-borne diseases (TBDs) [2]. *B. bovis* and *B. bigemina* are among the major tick-borne intraerythrocytic protozoan parasites affecting farm population globally [3]. Moreover, human babesiosis is attracting increasing attention as a worldwide emerging zoonosis. Humans are commonly infected by the bite of ixodid ticks. Infection of the human host can cause a very severe host-mediated pathology including fever, and hemolysis leading to anemia, hyperbilirubinuria, hemoglobinuria and possible organ failure [4].

In spite of the great importance of these parasites, little information is available about the epidemiology of them in KSA generally and in Aseer region particularly. [5] investigated the blood parasites of indigenous camels, sheep, goats and cattle in several localities in Saudi Arabia that are well isolated from any possible mixing with imported animals. Banaja and Ghandour [6] reviewed the parasitic protozoa, gastro-intestinal helminthes, extra-intestinal helminthes, naso-pharyngeal and dermal myiasis and Ticks and mited infestation in camels in Saudi Arabia. Al-Metenawy [7] examined blood samples from 301 sheep and 132 goats from AL-Qassim region, Saudi Arabia in the period from 1994 to 1997 for detection of blood parasites. Al-Khalifa *et al.* [8] microscopically examined blood samples for blood parasites from a total number of 700 camels, 548 sheep, 454 goats and 116 cattle from Aseer, Jazan, Riyadh, Tabouk, Eastern and Northern Frontiers (Saudi Arabia).

Traditionally, the microscopic detection of *Babesia* parasites has always been considered as the gold standard for the diagnosis of acute babesiosis [9]. However, molecular diagnosis was applied in the present study, since PCR-based assays have been widely used for the detection of *Babesia* parasites owing to their high specificity and sensitivity [10].

This work aimed to study the prevalence of *Babesia bovis* and *B. bigemina* infections in animals slaughtered in Abha and Khamis Mushait abattoirs, Aseer, KSA using polymerase chain reaction (PCR).

## MATERIALS AND METHODS

### Collection of Blood Samples

350 blood samples were collected from 30 camels, 4 cows, 136 goats and 180 sheep slaughtered in Abha and Khamis Mushait abattoirs, Aseer, KSA. Blood samples were collected in evacuated tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Blood was frozen at 70°C prior to DNA extraction.

### DNA extraction

The genomic DNA was extracted from the whole blood using a commercial kit (Bioingentech Genomic DNA Purification Kit) according to the manufacturer's instructions.

### Polymerase Chain Reaction (PCR)

The total genomic DNA samples from 350 animals were analyzed by PCR for detection of *B. bovis* and *B. bigemina*. VetPCR$^{TM}$ *B. bovis* and *B. bigemina*. Detection Kits (Bioingentech Ltd, Ref. VET-B002-48D and Ref. VET-B003-48D) were used. Each PCR reaction will be performed in a 13.5µl volume containing 2µl of the extracted DNA template, 5.5µl VetPCR$^{TM}$ *B. bovis* or *B. bigemina* premixture and 6µl DNase/RNase free water. The mixture was covered by 11µl mineral oil solution. The thermocycling conditions for PCR amplifications will be set as follows: initial denaturation in 2 min at 94◦C followed by 30 cycles (30 sec of denaturation at 94◦C, 30 sec of annealing at 57 ◦C, and 30 sec of extension at 72◦C) and final extension at 72◦C in 5 min. The PCR products will be then subjected to electrophoresis in 1.5% agarose gel containing ethidiumbromide and then will be visualized using UV gel documentation system. 100 bp Brig$^{TM}$ molecular weight marker was used.

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## RESULTS AND DISCUSSION

The PCR analysis using specific primers for *B. bovis* and *B. bigemina* revealed that *B. bovis* percentage of infection was 6.25, 0, 0.74 and 1.2 % for camels, cows, goats and sheep, respectively, diagnostic band of *B. bovis* appeared at 238bp (Figure 1).

In the present finding, *B. bovis* was reported for the first time in KSA. Neither *B. bovis* nor *B. bigemina* were investigated by Hussein *et al.* [5] in the blood of indigenous camels, sheep, goats and cattle in several localities in Saudi Arabia. Also, *B. bovis* and *B. bigemina* were not recorded by Banaja and Ghandour [6] in their review of parasites of camels in KSA; the protozoan parasites recorded by them were *Trypanosoma evansi*, *Sarcocystis cameli*, *Eimeria dromedarii*, *E. cameli*, *E. rajasthani* and *Thileria* spp. *Theileria hirci* was the only parasite recorded by Al-Metenawy [7] in the blood samples from 301 sheep and 132 goats from AL-Qassim region, Saudi Arabia. In addition, *B. bovis* was not detected by Al-Khalifa *et al.* [8] who examined blood samples for parasites from a total number of 700 camels, 548 sheep, 454 goats and 116 cattle from Aseer, Jazan, Riyadh, Tabouk, Eastern and Northern Frontiers (Saudi Arabia).

The detection of *B. bovis* for the first time in the KSA in the present study may be due to the sensitivity, specificity and accuracy of the diagnostic method used, since microscopic examination was the tool used in the previous Saudi studies. The major drawback associated with microscopic examination of blood parasites is the low sensitivity offered by the technique, thus making it difficult to detect parasites in blood smears during low parasitemia in the case of carrier animals [11].

The present recorded prevalence of *B. bovis* is lower than those reported in animals from Thailand by Cao *et al.* [12] since their results revealed a prevalence of 12 and 21 % for *B. bovis* and *B. bigemina*, respectively, by application of a nested PCR assays; in addation, Mtshali *et al.* [13] by using the same technique in cows from South Africa revealed that the overall prevalence was 35.5% and 76.1% for *B. bovis* and *B. bigemina*, respectively. The low *B. bovis* prevalence in the present work may be due to that the blood samples were collected from animals in official abattoirs and the owners of such animals were keen to provide healthy animals to the slaughterhouse, where it will be subject to inspection by the health authorities before and after slaughter process. In addition, the prevalence varies from region to region and various factors determine the occurrence of the TBDs including type of host, age, sex, breed, tick density, season, geographical area and management [14,15].

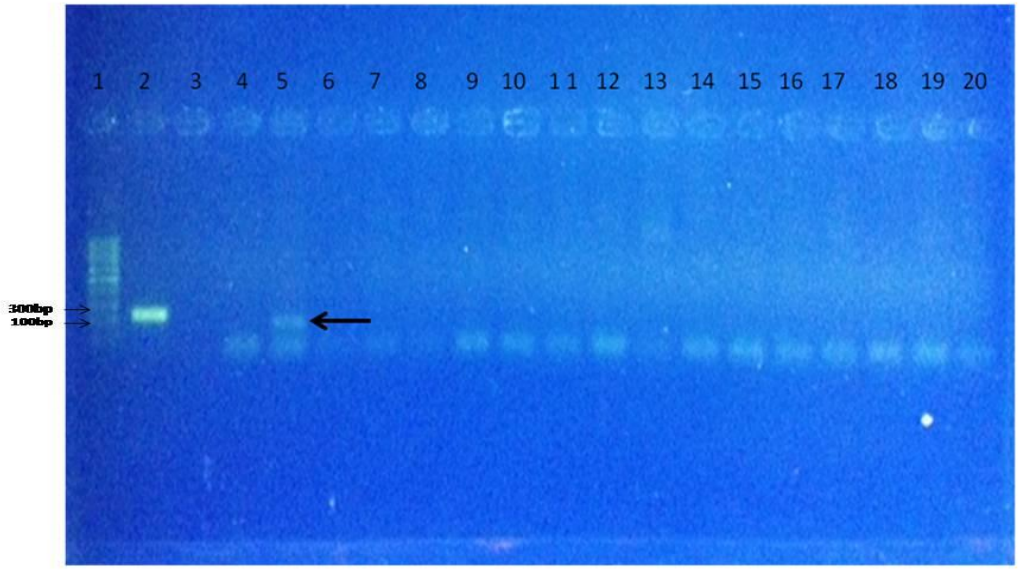


**Figure 1: PCR products using *B. bovis* specific primer 1. Lane 1: DNA marker; Lane 2: positive control; Lane 3: negative control; Lanes 4 to 8 samples from camels; Lanes 9 to 10 samples from cows; 11 to 15 samples from goats; Lanes 16 to 20 samples from sheep; arrow directed to positive sample.**

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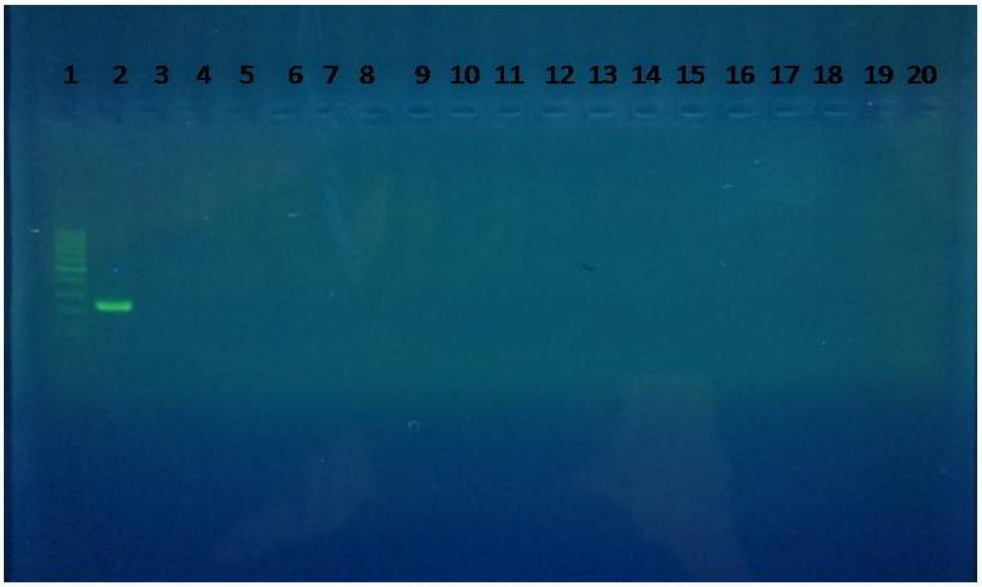


**Fig.2: PCR products using B. bigemina specific primer, Lane 1: DNA marker; Lane 2: positive control; Lane 3: negative control; Lanes 4 to 8 samples from camels; Lanes 9 to 10 samples from cows; 11 to 15 samples from goats; Lanes 16 to 20 samples from sheep.**

In the present study, all samples appeared negative to *B. bigemina* (Figure 2). In Saudi Arabia, this parasite was recorded only in cattle from Jazan Region by Al-Khalifa *et al.* [8]; the rate of infection was 6%. However, it wasn't recorded in Aseer, Riyadh, Tabouk, Eastern and Northern Frontiers, other regions of Saudi Arabia studied by the same authors. Al-Khalifa *et al.* [8] suggested that *B. bigemina* has been probably introduced with infected livestock infested with vector ticks imported from one of infected countries outside the Saudi Arabia. In addition, the vector of this parasite, the tick *Boophilus annulatus*, has been reported from cattle in Jazan by Al-Khalifa *et al.* [16] who suggested that this tick might have introduced its pathogen too into the Kingdom. The absence of *B. bigemina* in the present finding was in agreement with that of Hussein *et al.* [5], Banaja and Ghandour [6] and Al-Metenawy [7] who studied the blood parasites of farm animals in KSA.

These results provided important information about Babesiosis prevalence in Aseer region, KSA. In addition, the sensitive and accurate method of diagnosis used in this study will be helpful in large-scale monitoring of transmission foci in endemic areas and will be important for the long-term control of the disease.

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