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Molecular Diagnosis of Tick-Borne Haemoprotozoan Disease Agents in Ticks Collected from Sheep

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Abstract. Piroplasmosis, a tick-borne haemoprotozoan disease, is a major constraint for small ruminant's health and production in Asia, Africa and southern Europe. The main hosts of *Theileria lestoquardi* are sheep and goats. The infection is transmitted by ticks of the genus *Hyalomma*. The aim of this study was the molecular surveillance of *Theileria lestoquardi* occurring in *Hyalomma anatolicum anatolicum* ticks based on the study of the 18S rRNA gene. A total 100 ticks of *Hyalomma anatolicum anatolicum* species were collected from the sheep with the signs of theileriosis in clinical observations.The PCR results was positive for 59% tick samples with higher infection rate in the females ticks (67.3%) compared to the males ones (51.8%). Sequencing results, also, revealed that the ticks were infected with *Theileria lestoquardi* and *Theileria ovis*

Keywords: Sheep, Theileria lestoquard, *Hyalomma anatolicum anatolicum*, Piroplasmosis,

1. Introduction

Ticks transmit a greater variety of pathogenic micro-organisms than any other arthropod vector group. These include tick-borne protozoa and tick-borne bacteria of both medical and veterinary importance. Tick-borne protozoan diseases such as theileriosis and babesiosis pose important problems for the health and management of domestic ruminants in the tropics and subtropics[1]. From sheep-infecting *Theileria* parasites, *Theileria lestoquardi* and some *Theileria* species from North China are considered to be highly pathogenic [2], [3]. *Theileria lestoquardi* is an important pathogenic agent in Iran [4], Iraq [5] and India [6]. The diagnosis of tick-borne protozoa is based on the morphological examination - tick salivary gland staining, Giemsa-stained blood smears, serological assays and clinical symptoms. These conventional methods are often time consuming and less reliable. New molecular techniques have been developed recently including polymerase chain reaction (PCR) or reverse line blot (RLB) [7], [8], [9], [10] which allow the direct, specific, sensitive and simultaneous detection and differentiation of different pathogens.

In the present study the tick samples collected from Fars province – an area with the highest level of sheep theileriosis cases in Iran and more than 90% of sheep theileriosis vaccine consumption [11].The aim of this research was the molecular diagnosis of protozoa occurring in *Hyalomma anatolicum anatolicum* infected sheep based on the study of the 18S rRNA gene.

2. 2.Materials and Methods

One hundred ticks of *Hyalomma anatolicum anatolicum* species were collected from the sheep with the signs of theileriosis in clinical observations. All samples were collected from veterinary clinics of Shiraz

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suburb. The ticks were identified and maintained in tubes containing ethanol until use for examination for protozoa. DNA was extracted according to the manufacturer's protocol from ticks using the QIAamp extraction kit (Qiagen). For PCR amplification of the 18S rRNA gene primers RLB F2 and RLB R2 were used as described by Gubbels et al. [12]. The reaction was incubated at 94°C for 10 min then the thermal cycle reaction programmer was as follows: 94°C for 20 sec, 67°C for 30 sec and 72 °C for 30 sec for two cycles. During the subsequent two-cycle sets the annealing temperature was lowered by 2°C until it reach 59°C following a traditional touch-down programmer. Then for the next 30 cycles annealing temperature was 57 °C. The PCR reaction was ended by a final extension at 72°C for 5 min. PCR products were visualized on 1.2% agarose gel stained with ethidium bromide and under UV light using a Bioimagine system SYNGENE. After amplification the generated DNA fragments were sequenced and compared with nucleotide databases (GenBank, NCBI).

3. Results

PCR results indicated a positive infection of 59% protozoa in ticks with the higher rate of infection for females compared to males (Table 1).

Sequencing results from 20 ticks showed that 18 ticks were infected with *T. lestoquardi* (98% - 99% identities with *T. lestoquardi,* GeneBank accession number GU233776) and two ticks with *Theileria ovis* (97-99% identities with *Theileria ovis.,* GeneBank accession numbers FJ752026).

4. Discussion

In the present study all ticks samples were collected from clinical cases in veterinary practices so higher infection rates is expected than screening from the field .The PCR results showed 67.3% and 51.8% positivity in female and male ticks respectively ,which is in agreement with similar work by Razmi et al. [13]. Sequencing analysis showed that *T. lestoquardi* was the dominant species in Shiraz suburb and this finding is in accordance with pervious report that Fars province has highest level of sheep theileriosis cases in Iran [11].

These findings reconfirmed previous studies which mentioned ovine theileriosis due to Theileria lestoquardi is distributed in south and south-east regions and T. ovis infection is widespread all over the country [14]. It is also important to mention that the *Theileria ovis* found in this study is the first report of this protozoa from Fars province .

5. References

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Table 1 : Tick samples from sheep and their PCR positivity for protozoa

Region	Female	Male	Total
Shiraz	31/46 67.3%	28/54 51.8	59/100 59%

Number of positive samples/Number of total samples