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Study on prevalence of blood parasites of sheep and detection of their vectors using methyl green pyronin in Varamin, Iran

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ABSTRACT

Theileria and Babesia are some major pathogens in animals which can transmit by ticks. They cause significant economic losses in tropical and sub-tropical regions of the world. Climate variability and its changes in different regions can change the parasites fauna and their vectors. So the regular study on their prevalence can be very useful for control and prevention strategies. For this purpose a total of 300 sheep with clinical sign (Fever, jaundice and anemia) in Varamin were tested for presence of Theileria and Babesia using Giemsa staining. Also the present ticks on these animals were collected and identified using the identification keys, and then salivary glands from some ticks collected from positive animals for presence of pathogens in blood were dissected and stained using Methyl Green Pyronin. Six tick species were identified as: Hyalomma anatolicum, H.asiaticum, H.marginayum, Rhipicephalus turanicus, Rh. bursa and Rh. sanguineus. Rhipicephalus female was the most frequent tick (51.87%) and Rh. sanguineus was the minor species (0.58%). In blood smears examination, Theileria was the only parasite which was seen with 2% frequency. None of the stained salivary glands had positive reaction to Methyl Green Pyronin that showed, positive animals for presence of Theileria, infected by another ticks previously. It seems that the life cycle of Theileria has not completed in collected ticks because of shortage of feeding period.

Key words: Blood Parasites, Sheep, Tick Salivary Gland, Methyl Green Pyronin.

INTRODUCTION

Piroplasmosis caused by tick-borne hemoprotozoan parasites including Theileria spp. and Babesia spp. is a major problem in small ruminants. Theileriosis and babesiosis are parasitic diseases of economic importance and significantly affect the international trade of animals [1-4]. Generally, the diagnosis of ovine piroplasmosis is based on morphological examination of blood smears and clinical symptoms.

Detection of Theileria sporoblast in the salivary gland of Hyalomma anatolicum has been carried out by various staining techniques by several workers [5-9] for epidemiological studies. However, in Iran many works have been carried out but no such study has been conducted in Varamin. The detection of Theileria infection rates and intensity

in the vector ticks is an important component in the study of epidemiology as it quantifies the flow of infection in nature.

MATERIALS AND METHODS

Collection of sample

This study was carried out in order to investigate the prevalence of sheep's blood parasites in Varamin, Iran, during spring and summer 2012. A total 300 thin blood smears were collected from flocks with a history of tick infestation, relapse of fever and anemia. Clinical signs were also recorded. 171 ticks were collected manually from the head, ears, axial, abdomen and genital regions in labeled bottles. Adult ticks were morphologically examined under the stereomicroscope (Kyowa, Tokyo) and identified according to Estrada-Pena et al [10].

Microscopic examination

Two thin blood smears were prepared from each sample. These smears were air dried, fixed in methanol for 3 min and stained in Giemsa diluted at 5% with buffer solution for 45 min and examined at 100× oil immersion objective lens of a Nikon SE light microscope. At least twenty microscopic fields of each slide were examined for search of blood parasites.

Staining of salivary glands

Dissection of ticks was done according to the method of Purnell and Joyner [11]. The salivary glands of dissected ticks were harvested and stained by Methyl Green Pyronin stain as per the method of Irvin et al [12].The salivary glands were fixed by dipping the slide in Cornoy's fixative for 5 min. The slides were then rinsed in 70% alcohol for 2 min followed by distilled water for 2 min and then air-dried. They were next immersed in Methyl Green Pyronin staining solution for 7 min and rinsed in distilled water and air-dried. Lastly when completely dry, the slides were dipped briefly in xylene before mounting in Depox. The prevalence and the intensity of infection were determined for each tick species and sex.

Statistical analysis

The data were analyzed using chi-square test. Statistical calculations were performed using SPSS (16.0) software. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Microscopic examination

In the microscopic examination of blood smears, the parasitemia ranged an average of 0.07 to 0.1%. Piroplasmos, detected inside the red blood cells were oval and ring forms. Of the 300 blood smears examined, 6 (2%) were positive for these piroplasmas. Abnormalities in erythrocytes including anisocytosis, poikilocytosis and basophilic strippling were evident.

Tick species infesting the sheep and their numbers

A total of 171 adult ticks belonging to two species were collected from the sheep (n = 300) during the period under review. These are summarized in Table 1. Rhipicephalus spp. was generally the dominant species. None of the stained salivary glands had positive reaction to Methyl Green Pyronin.

TABLE 1- Numbers and percentage of prevalence of ticks in the study area in Varamin

Species / Tick	*Hyalomma anatolicum*	*Hyalomma asiaticum*	*Hyalomma marginatum*	*Rhipicephalus turanicus*	*Rhipicephalus bursa*	*Rhipicephalus sanguineus*
Number	3	28	9	127	2	1
Percentage	1.75%	16.37%	5.26%	74.26%	1.16%	0.58%

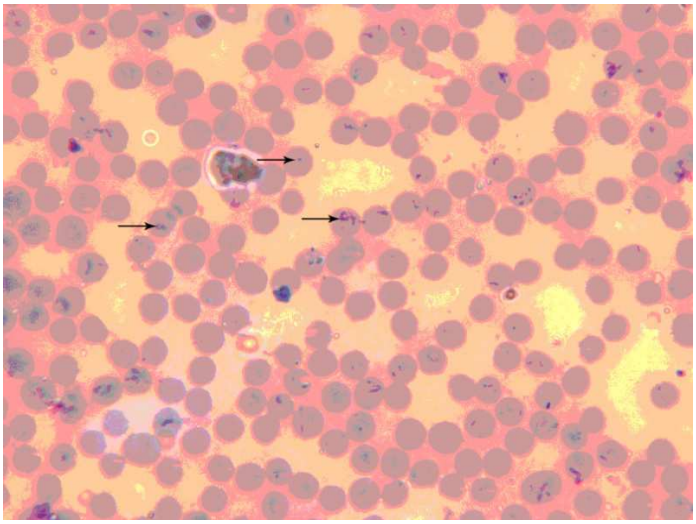

Fig 1. Theileria spp. inside red blood cell (×1800)

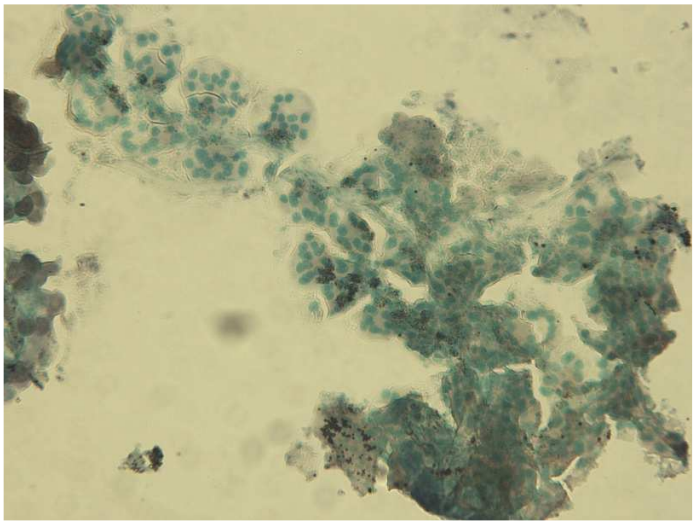

Fig 2. Methyl Green Pyronin stained salivary gland (×10)

Piroplasmosis is severe tick borne protozoan disease in tropical and subtropical regions all over the world. The causative agents of piroplasmosis are belonging to the genera of Theileria and Babesia [13]. Ovine piroplasmosis including both theileriosis and babesiosis are high pathogen. Theileria lestoquardi (Theileria hirci) and Theileria ovis are the causal agents of small ruminants theileriosis which widespread throughout Iran [14,15]. The causal agent of malignant sheep and goat theileriosis is T.lestuquardi with the more prevalence in south and east provinces of Iran [14,16,17]. Babesia parasites are not only cause significant loss in livestock industry, but also considerable in the public health problem [18-20]. Babesia ovis is considered highly pathogenic in sheep that reported from different parts of Iran [14,20]. Specific identification of Theileria and Babesia species in tick vectors is important for the epidemiological studies and development of effective control and treatment strategies.

In the present study carried out in the Varamin, Rhipicephalus turanicus, Rh. Bursa, Rh. Sanguineus, H. anatolicum, H. asiaticum and H. marginatum were recovered from sheep. This study showed that Rhipicephalus was the major tick species of sheep in the Varamin. Rh. sanguineus was less frequently seen on sheep. None of the stained salivary glands had positive reaction to Methyl Green Pyronin that showed, positive animals for presence of Theileria, infected by another ticks previously. It seems that the life cycle of Theileria has not completed in collected ticks because of shortage of feeding period.

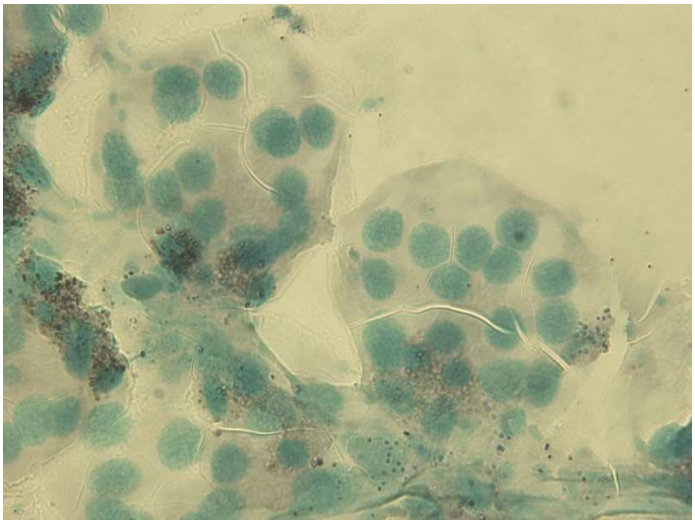

Fig 3. Methyl Green Pyronin stained salivary gland (×40)

CONCLUSION

In conclusion, the results of the present study indicate the presence of piroplasmosis in Varamin. Additional molecular studies are essential for determining effective control strategies and developing vaccines against these protozoan parasites.

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