

Infectivity of *Theileria annulata* in *Hyalomma* ticks of Eastern Terai districts, Nepal

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Abstract

The study was conducted to assess the prevalence of *Theileria* infection in *Hyalomma* tick vectors collected from cattle host to record the natural infection level of theilerial parasite in the field condition. A total of 174 male and 298 female of *Hyalomma marginatum issaci* ticks were collected from three districts Sunsari, Morang and Jhapa of Eastern Terai of Nepal during 2004-2005. These *Hyalomma* ticks were dissected and their salivary glands were stained with Methyl Green Pyronin (MGP) stain. 472 *Hyalomma* ticks were screened and 114 (24.15%) were found positive for *Theileria*. The percent of *Theileria* positive ticks were 8.62, 27.35 and 20.63 from Sunsari, Morang and Jhapa respectively. The prevalence, abundance and intensity of *Theileria annulata* infection were higher in female *Hyalomma* ticks (27.18%, 6.61 and 24.31 respectively) than male *Hyalomma* ticks (18.97%, 3.60 and 19.00 respectively).

Keywords: Cattle parasite, Intensity, Methyl Green Pyronin (MGP) stain, Salivary gland

Introduction

Bovine tropical theileriosis caused by *Theileria annulata* and transmitted by *Hyalomma* sp. is an economically important disease of cross-bred cattle which causes heavy economic loss in terms of high morbidity and mortality, loss of productivity and indirect losses due to cost of control measures. This posed a major challenge in the dairy pockets of Eastern Terai Region of Nepal where both the vectors as well as the parasites have been identified (Acharya and Pradhan 1996, Shrestha and Singh 2000 and RVL 2001/02). Occurrence of theileriosis shows

seasonality and the disease is mostly observed in summer from March to October. This is related to the activity of ticks' population which varies with climatic conditions viz. temperature, rainfall and humidity. Epidemiological studies in India have shown that young calves are highly susceptible to the disease. Depending upon the quantum of infection, these calves either suffer severely and die of the disease or react and recover. Breakdown of the preimmune status following stress of pregnancy, parturition, lactation, inter-current disease conditions and environmental factors is reportedly responsible for the occurrence of clinical form of the disease amongst adult exotic and crossbred cattle. Over 250 million cattle, in endemic areas were estimated to be at risk from *T.annulata* infection (Robinson 1982). Because of different cattle breeds, different tick vectors with different biology from one region to another and regional and seasonal variations, the epidemiology of tropical theileriosis is not the same everywhere. The detection of *Theileria* infection rate and intensity of infection in the vector ticks is an important component in the study of epidemiology of theileriosis. Work on this aspect was initiated with *T. parva* in Kenya and with *T.annulata* in Sudan (Walker et al. 1983) and India (Sangwan et al. 1986).

Materials and Methods

This study was conducted in Sunsari, Morang and Jhapa districts of Eastern Terai of Nepal during May 2004 to June 2005. *Hyalomma* ticks were collected manually from different part of cattle body. These ticks were preserved in 70% alcohol containing 5% glycerin. The preserved ticks were examined for their morphological characteristics under stereoscopic microscope and identified according to the figures and key described by Acarology Division, IMR 1995, Kaiser and Hoogstraal 1964, Morel 1989 and MAFF 1986. The *Hyalomma* ticks were dissected under a stereoscopic microscope according to the procedure of Blewett and Branagan (1973). Salivary glands were stained by Methyl Green Pyronin (MGP) staining method described by Irvin et al. 1981. Fixed in Cornoy's fixative, cleared in Xylene and mounting in DPX.

For assessment of infectivity rates of *Hyalomma* ticks, number of infected acini per tick were counted and recorded for both male and female ticks on the basis of the characteristics of the infected acini described by Walker et al. (1979). The degree of infection with *Theileria annulata* were expressed as prevalence [(number of infected ticks/number of ticks examined)

x100], abundance (number of infected acini/number of ticks examined) and intensity (number of infected acini/number of ticks infected) as per Margolis et al. (1982).

Statistical Analysis

The infectivity of bovine tropical theileriosis was analysed using chi-square (χ^2) test by F. Yates correction method.

Results

Out of a total of 472 *Hyalomma* ticks screened for the presence of *Theileria*, 114 ticks were positive for *Theileria*. Thus the general prevalence was 24.15% of the total ticks screened in the Eastern Terai of Nepal. The theilerial mass/sporoblast in the infected cell cytoplasm took greenish blue colour (Plate 1). The increase in size of the infected acinus showed great variations. In *Theileria* positive hypertrophied acini, the normal acinar cell nuclei were pushed towards one side. Hypertrophied nucleus of the infected cell was either in the center of the *Theileria* mass or at the periphery (Plate 1).

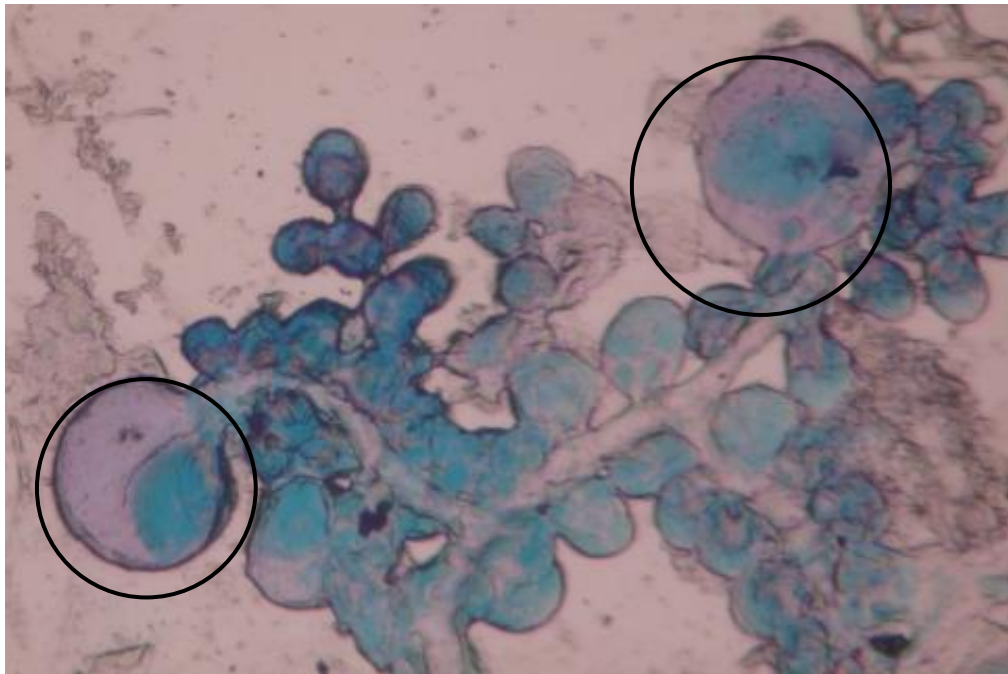


Plate 1. MGP stained salivary gland showing *Theileria* sporoblast infected acini harvested from *Hyalomma* tick (1000x)

In Eastern Terai Region a total of 58 *Hyalomma* ticks were examined from Sunsari district and 5 (8.62%) of them were *Theileria* positive. From Morang district, out of 351 *Hyalomma* ticks examined 96 (27.35%) were found positive. Of a total of 63 *Hyalomma* ticks examined from Jhapa district, 13 (20.63%) showed *Theileria* positivity (Figure 1).

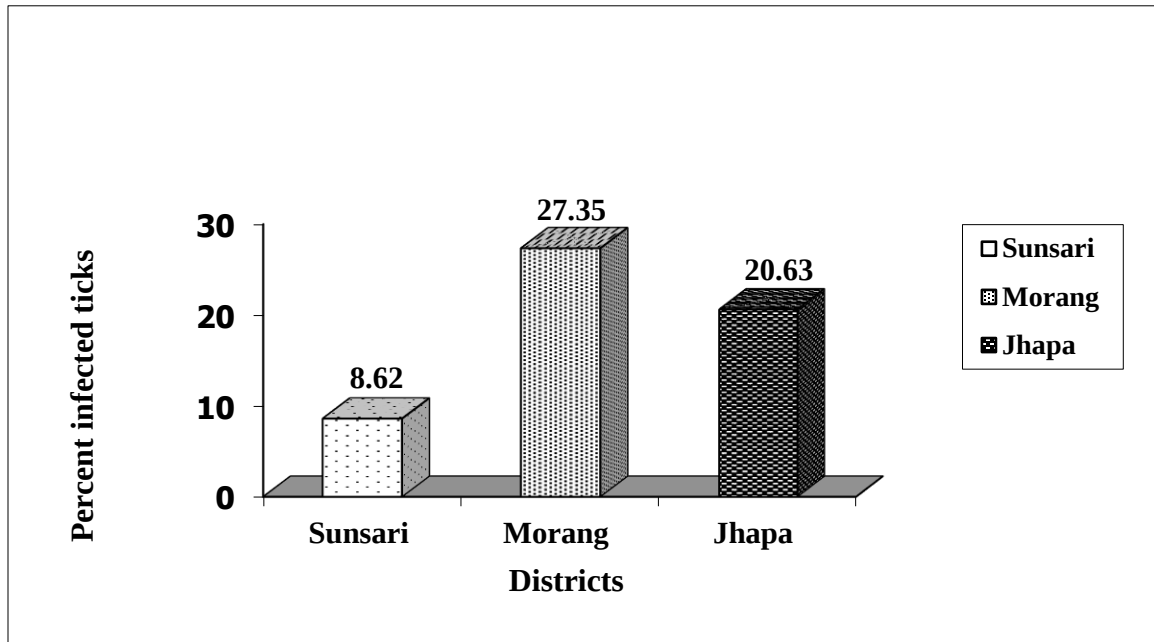


Figure 1. *Theileria* infection rate in *Hyalomma* ticks of Eastern Terai of Nepal

The prevalence, abundance and intensity of *Theileria* infection were found to be higher in female ticks (27.18%, 6.61 and 24.31 respectively) than male ticks (18.97%, 3.60 and 19 respectively) (Table 1).

Table 1. Prevalence of *Theileria annulata* infection in *Hyalomma marginatum issaci* of Eastern Terai of Nepal

Category	No. of ticks examined	No. of infected ticks	Total infected acini	Degree of salivary gland infection		
				Prevalence ^a %	Abundance ^b	Intensity ^c
Sex of ticks						
Male	174	33	627	18.97	3.60	19.00
Female	298	81	1969	27.18	6.61	24.31
χ^2 Value	-	4.048	-	-	-	-

^aTotal no. of infected ticks/total no. of ticks examined $\times 100$, ^bTotal no. of infected acini/total no. of ticks examined, ^cTotal no. of infected acini/total no. of infected ticks.

Discussion

The present study indicates that theileriosis is now endemic in Morang district. Similar findings were reported by the studies made in the context of Eastern Terai of Nepal in the past (Acharya and Pradhan 1996, Shrestha and Singh 2000). Prevalence of the theileriosis is assumed to be much higher than that reported on the basis of clinical cases. Animals were treated on the basis of clinical symptoms alone in the field condition and many cases remain undiagnosed. Cases of theileriosis occurred between the months of March and October. It is a vector borne disease and a peak of tick activities during hot and humid climate of summer and rainy seasons appears to be responsible for this seasonal increase in clinical form of theileriosis. Observations made by Flatch and Ouhelli (1992) were similar and Radostits et al. (1994) also pointed out the importance of air temperature in variation of tick population and thus the seasonal incidence of the disease. Occurrence of 24.15% of the theileriosis as found in the present study is consistent with the epidemiological findings from RVL 2001/2002, and from India which had shown the high susceptibility in small age group (Beniwal et al. 1997). *Hyalomma marginatum issaci* identified in this study is the specific vector of bovine tropical theileriosis. With the change in ecological, agronomical and managemental practices, the microclimate and ecological niche of livestock ticks are undergoing changes leading to disturbances in tick populations. In this study, almost in all animals, the number of tick infestation per animal was more than fifty. *Hyalomma marginatum issaci*, the vector tick for theileriosis, was identified from cattle of three districts Sunsari, Morang and Jhapa of Eastern Terai, Nepal. In the presence of parasites as well as the vectors, it can be said that Eastern Terai region is endemic for theileriosis. However, very scanty work has been done regarding quantitative epidemiology of bovine tropical theileriosis in Nepal. Detection of theilerial sporoblasts in the salivary glands of vector ticks are the most useful tools for the epidemiological studies of theileriosis. The tick collection methods employed in the present study were found adequate and feasible for the quantitative assessment of *Theileria* infection in the field. It is very difficult to guess the physiological age of an adult male tick as its size does not vary appreciably with the age and unlike female ticks it can feed repeatedly on the host for a long period. Due to this factor, a direct collection of engorging ticks from the host for theilerial assessment is not preferred. However, several workers (Bouattour et al. 1996, Kumar 2000) have dissected engorging ticks collected from

the hosts as it is far more convenient and still accurate for the female ticks. Whole salivary gland staining with MGP worked satisfactory for assessing *Theileria* infectivity in ticks. The most consistent diagnostic characteristics of the presence of *Theileria* masses i.e. sporoblasts in the salivary gland acinus as indicated by the hypertrophy of the acinus and of infected acinar cell nucleus along with the presence of diffuse theilerial mass in the cytoplasm observed in this study, confirmed the earlier observations of this feature (Blewett and Branagan 1973, Walker et al. 1979, Sangwan et al. 1986). RVL (1996) was also recorded 21(43.75%) positive cases of *T. annulata* of the total 48 cases. In the present study, 114 (24.15%) out of 472 *Theileria* positive cases was recorded. The prevalence percent, abundance and intensity of theilerial infection in *Hyalomma* ticks were higher in female ticks than male ticks. This finding will contribute to a better knowledge of the epidemiology of *T. annulata* infection in Eastern Terai of Nepal.

Conclusion

Bovine tropical theileriosis has remained a major health hazard for the exotic and crossbred cattle since the introduction of large scale crossbreeding of native cattle in Nepal with the dairy breeds. In addition to the great economic losses, the disease is also inhibiting the potential for milk production by making farmers reluctant to opt for cross breeding of their cattle. Although bovine tropical theileriosis is widespread in Eastern Terai of Nepal and is a serious challenge to a livestock development programmes, its epidemiology has not been adequately studied. Study of prevalence of *Theileria* both in cattle and in the vector ticks is an important component to know the epidemiology of theileriosis. The epidemiology of the disease has not been studied so far in the Eastern Terai of Nepal where the vector ticks are found in abundance. Out of a total of 472 ticks screened for the presence of *Theileria*, 114 (24.15%) ticks were positive for *Theileria*. In order to know the level of *Theileria* infection in *Hyalomma* ticks in the Eastern Terai of Nepal, frequency distribution of *Theileria* positive acini per positive tick was worked out. 85.09 percent ticks showed one to five positive acini and 14.91 percent ticks showed more than five positive acini.

Recommendations

Based on the research findings, following recommendations could be made:

- * Infectivity of *T. annulata* in *Hyalomma* ticks was high in the Eastern Terai of Nepal. The occurrence of clinical cases of theileriosis in cattle of the Eastern Terai of Nepal may be more. So, further investigation should be carried out.
- * Infectivity of *T. annulata* was higher in female ticks than male ticks in all three districts of the Eastern Terai of Nepal. This study should be continued.
- * The sero-prevalence study of theileriosis in cattle should be carried out.
- * Introduction of susceptible crossbred cattle in Eastern Terai of Nepal needs rethinking.
- * This study should be carried out again to find the effect of *T. annulata* in economy status of Nepal.

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