Prevalence of *Theileria annulata* infection in *Hyalomma anatolicum anatolicum* in Punjab state, India

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Abstract The study was conducted to assess the prevalence of *Theileria* infection in tick vectors collected from healthy animals to record the natural infection level of theilerial parasite in the field condition. A total of 156 male and 110 semi-engorged female of *Hyalomma anatolicum anatolicum* were collected from cattle and buffaloes of two different agro climatic zones of Punjab, dissected and their salivary glands were stained with Methyl Green Pyronin (MGP) stain. The prevalence, intensity and abundance of *Theileria annulata* infection were higher in female ticks (15.45%, 5.08 and 32.88, respectively) than male ticks (8.97%, 1.69 and 18.86, respectively). The ticks collected from cattle had a higher prevalence, abundance and intensity (15.15%, 5.38 and 35.53, respectively) of *T. annulata* infection than the ticks collected from buffaloes (9.58%, 1.74 and 18.13, respectively). Hot and dry climate favored the development of *T. annulata* sporozoites in *H. a. anatolicum* ticks as the prevalence, abundance and intensity of *T. annulata* infection were higher in ticks collected from Western semi arid zone of Punjab as compared to those collected from the central plain zone with hot and humid climate.

Keywords *Hyalomma anatolicum anatolicum* · Methyl Green Pyronin (MGP) stain · Punjab · *Theileria annulata*

Introduction

Bovine tropical theileriosis transmitted by *Hyalomma anatolicum anatolicum* is an economically important disease of cross-bred cattle in India which causes heavy economic losses in terms of high morbidity, mortality and reduced production in recovered animals. In India around 10 million cattle are at risk for tropical theileriosis with an annual economic loss US \$800 million (Brown 1997). The determination of the pattern of *Theileria* challenge in the field through tick studies is a crucial rewarding approach to *Theileria* epidemiology. Detection of *Theileria* sporoblast in the salivary gland of *H. a. anatolicum* has been carried out by various staining techniques by several workers (Blewett and Branagan 1973; Walker et al. 1979, 1981; Leitch and Young 1981; Buscher and Tanguis 1986) for epidemiological studies. However, in India only limited work has been carried out in Haryana (Sangwan et al. 1986), Uttar Pradesh (Das and Ray 2003) but no such study has been conducted in Punjab state. 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

Geographical information

Amongst the six agro-climatic zones of Punjab state, two distinct climatic zones viz. Central plain (districts Ludhiana) and Western semi arid (district Bathinda) have been selected for the study. Western semi arid region the most hot and dry zone whereas, the central plain zone receive

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higher rainfall with humid climate. The average monthly rainfall of these two districts is depicted in Fig. 1.

Collection of ticks

Partially fed adult *H. a. anatolicum* ticks (identified as per the keys of Kaiser and Hoogstraal 1964) of both sexes were collected from healthy cattle and buffaloes from different organized and unorganized dairy farms of various villages of Ludhiana (Central plain zone) and Bathinda districts (Western semi arid region) of Punjab, India. The collection was done from January 2009 to June 2009. Those ticks were then transported to laboratory and transferred to dessicator in which 80–85% relative humidity was maintained using 10% KOH (Solomon 1951). The dessicator was kept in incubator at a constant temperature at 28°C.

Staining of salivary glands

Dissection of ticks was done according to the method of Purnell and Joyner (1968). The salivary glands of dissected ticks were harvested and stained by Methyl Green Pyronin (MGP) stain as per the method of Irvin et al. (1981). 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 DPX.

Interpretation of the stained salivary glands

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 *T. annulata* were expressed as prevalence [(number of infected ticks/number of ticks examined) $\times$ 100], abundance (number of parasite

masses/number of ticks examined) and intensity (number of parasite masses/number of ticks infected) as per Margolis et al. (1982).

Statistical analysis

A χ^2 test was used to assess statistical association between the categorical variables by SPSS 13.0 software.

Results and discussion

A total of 266 adult *H. a. anatolicum* ticks of both the sexes collected, out of which 31 ticks were found positive for *Theileria* sporoblast with a total of 823 positive acini. In most of the cases the positive acini were hypertrophied and were almost double in size than the normal acini. Interestingly few positive ticks were found to have acini normal in size but the nucleus of those acini were hypertrophied and intense green in colour while the parasite stain blue to purple (Fig. 2). The overall prevalence of *Theileria* in *H. a. anatolicum* was 11.65% and the abundance and intensity of infection were 3.09 and 26.55, respectively. Among a total of 156 adult male *H. a. anatolicum* ticks 14 ticks were found positive for *Theileria* sporoblast with 264 infected acini. The prevalence of *Theileria* infection in adult male *H. a. anatolicum* ticks was 8.97% with an abundance and intensity of 1.69 and 18.86, respectively. Similarly, out of 110 adult female ticks 17 ticks were found positive for *Theileria* sporoblast with 559 infected acini with the prevalence of 15.45%. The abundance and intensity of infection were 3.09 and 26.55, respectively (Table 1).

The prevalence rate of *Theileria* infection was higher in female ticks than in males but the difference was not significant ($P > 0.05$) probably because of smaller sample size. The mean intensity of *Theileria* infection in female and male ticks of *H. a. anatolicum* species was different

Fig. 1 Average monthly rainfall of district Ludhiana and Bathinda

Fig. 2 Methyl Green Pyronin stained salivary gland showing *Theileria* sporoblast infected acini harvested from *H. a. anatolicum*

Table 1 Prevalence of *Theileria annulata* infection in *H. a. anatolicum* of Punjab state, India

Category	No. of ticks examined	No. of infected ticks	Total infected acini	Degree of salivary gland infection		
				Prevalence % ^a	Abundance ^b	Intensity ^c
<i>Sex of ticks</i>						
Male	156	14	264	8.97	1.69	18.86
Female	110	17	559	15.45	5.08	32.88
χ^2 value	–	2.63	–	–	–	–
<i>Host</i>						
Cattle	99	15	533	15.15	5.38	35.53
Buffalo	167	16	290	9.58	1.74	18.13
χ^2 value	–	1.87	–	–	–	–
<i>Zones</i>						
Central plain	190	18	427	9.47	2.24	23.72
Western semi arid	76	13	396	17.11	5.21	30.46
χ^2 value	–	3.07	–	–	–	–
Total	266	31	823	11.65	3.09	26.55

^a Total no. of infected ticks/total no. of ticks examined $\times$ 100^b Total no. of infected acini/total no. of ticks examined^c Total no. of infected acini/total no. of infected ticks

with female ticks having higher intensity than the male. The role of the sex of the tick in *Theileria* transmission has previously been emphasized and it has been reported that both the prevalence and the intensity of *Theileria* infection were significantly higher in female ticks than in male ticks (Walker et al. 1983; Buscher and Tanguis 1986; Sangwan et al. 1989; Sayin et al. 2003; Aktas et al. 2004). The female ticks play a more important role in the transmission of *Theileria* infection as they had more abundance of type III acini than male ticks and *Theileria* parasites were only detected in type III acini (Irvin et al. 1981; Pipano et al. 1982; Young et al. 1983). Hence the abundance of type III acini in female ticks favors the development of *Theileria annulata* thus causing an increased incidence of infection in female ticks.

The prevalence, abundance and intensity of *Theileria* infection in *H. a. anatolicum* collected from cattle were 15.15%, 5.38 and 35.53 whereas in those collected from buffaloes were 9.58%, 1.74 and 18.13, respectively (Table 1). The prevalence, abundance and intensity of *Theileria annulata* sporoblast in *H. a. anatolicum* ticks collected from cattle were higher than in those ticks collected from buffalo and was also reported earlier by Sangwan et al. (1986). This finding attribute to the fact that the cross bred cattle are at a high risk and a preferred host of bovine tropical theileriosis as compared to buffaloes.

The prevalence, abundance and intensity of *Theileria* infection in *H. a. anatolicum* collected from Central plain zones was 9.47%, 2.27 and 23.72 whereas from Western Semi arid zone was 17.11%, 5.21 and 30.46, respectively (Table 1). The comparative infection rates of *Theileria* sp.

in *Hyalomma* sp. ticks in arid Rajasthan and semi-arid Haryana have been studied by Ram et al. (2004). The per cent prevalence of *Theileria* sp. infection in *Hyalomma* sp. ticks in Rajasthan was 29.85, as compared to 13.62 in Haryana. Similarly, theilerial intensity (18.50) and abundance (5.52) in the ticks collected from Rajasthan were much higher than the respective values of 11.46 and 1.56 in Haryana. Similar findings were recorded in the present study and ticks from drier areas had higher infection rates hence, the semi arid and arid region provide more favourable conditions for the development of *Theileria* organism in the vector tick as also reported earlier (Young and Leitch 1981; Sangwan et al. 1994; Ram et al. 2004; Sangwan 2007). This suggested that environmental temperature, humidity or some diurnal temperature rhyme or prolonged photoperiodicity have a positive correlation with the development of *Theileria* parasite in ticks (Young and Leitch 1981; Kamio et al. 1990).

The determination of the pattern of *Theileria* challenge in the field through tick studies is a crucial rewarding approach to *Theileria* epidemiology. In the present study, cattle and buffaloes from which ticks were collected, were free from symptoms of clinical theileriosis and were randomly selected from healthy animals. Blood smear examination for detection of *T. annulata* infection in these animals is not conclusive as these techniques fail to detect the carrier stage with very low parasitaemia (Friedhoff and Bose 1994). There was a low abundance of *Theileria* infection in *H. a. anatolicum* ticks collected from the field which suggests that this helps in establishing a stable enzootic condition of theleriosis and thus prevents

susceptible animals from the clinical disease in the field. This feature was also observed in *Rhipicephalus appendiculatus* ticks infected with *T. parva* in Africa (Young et al. 1986) and *Haemaphysalis longicornis* ticks infected with *T. sergenti* in Japan (Fujisaki and Kamio 1988; Kamio et al. 1990). It was concluded that in endemic areas, ticks would usually imbibe the minimal possible dose of *Theileria* from cattle to maintain the infection in their salivary gland. Also hot and dry (semi arid) environmental conditions are more suited for the development and maintenance of *T. annulata* infection in the vector tick.

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References

- Aktas M, Dumanli N, Angin M (2004) Cattle infestation by *Hyalomma* ticks and prevalence of *Theileria* in *Hyalomma* species in the east of Turkey. *Vet Parasitol* 119:1–8
- Blewett DA, Branagan O (1973) The demonstration of *Theileria parva* infection in intact *Rhipicephalus appendiculatus* salivary glands. *Trop Anim Health Prod* 5:27–34
- Brown CGD (1997) Dynamics and impact of tick-borne diseases of cattle. *Trop Anim Health Prod* 29:1S–3S
- Buscher G, Tangus J (1986) Quantitative studies on *Theileria* in the salivary glands of *Rhipicephalus appendiculatus* adults: search for conditions for high infection. *Int J Parasitol* 16:121–129
- Das G, Ray D (2003) PCR- based detection of *Theileria annulata* in ticks collected from cattle of West Bengal India. *J Vet Parasitol* 17:11–14
- Friedhoff K, Bose R (1994) Recent developments in diagnostics of some tick-borne diseases. In: Uilenberg G, Permin A, Hansen JW (eds) Use of applicable biotechnological methods for diagnosing haemoparasites. Proceedings of the expert consultation, Merida, Mexico, 4–6 October 1993. Food and Agriculture Organisation of the United Nations (FAO), Rome, Italy, pp 46–57
- Fujisaki K, Kamio T (1988) The effect of constant temperatures on *Theileria sergenti* infection in salivary glands of nymphal *Haemaphysalis longicornis*. *Jpn J Vet Sci* 50:529–536
- Irvin AD, Boarer CDH, Dobbelaere DAE, Mohan SM, Marake R, Ocama JGR (1981) Monitoring *Theileria parva* infection in adult *Rhipicephalus appendiculatus* ticks. *Parasitology* 82:137–147
- Kaiser MN, Hoogstraal H (1964) The *Hyalomma* ticks (Ixodoidea, Ixodidae) of Pakistan, India and Ceylon with keys to subgenera and species. *Acarologia* 6:257–286
- Kamio T, Ito Y, Fujisaki K, Minami T (1990) Infection rates of *Theileria sergenti* in *Haemaphysalis longicornis* ticks collected from the field in Japan. *Jpn J Vet Sci* 52:43–48
- Leitch BL, Young AS (1981) *Theileria* infection in *Rhipicephalus appendiculatus* ticks collected in the field. In: Irvin AD, Cunningham MP, Young AD (eds) Advances in the control of theileriosis. Martinus Nijhoff, The Hague, pp 63–65
- Margolis L, Esch GW, Holmes JC, Kuris AM, Schad GA (1982) The use of ecological terms in parasitology (Report of an ad hoc committee of the American Society of Parasitologists). *J Parasitol* 68:131–133
- Pipano E, Samish M, Krigel Y (1982) Relative infectivity of *Theileria annulata* (Dschunkowsky and Luhs, 1904) stabilate derived from female and male *Hyalomma excavatum* (Koch, 1844) ticks. *Vet Parasitol* 10:21–27
- Purnell RE, Joyner LP (1968) The development of *Theileria parva* in the salivary glands of the tick *Rhipicephalus appendiculatus*. *Parasitology* 58:725–732
- Ram R, Gupta SK, Sangwan AK (2004) Comparative infection rates of *Theileria annulata* in *Hyalomma anatolicum anatolicum* ticks in arid and semi-arid regions of north-west India. *Trop Anim Health Prod* 18:109–114
- Sangwan AK (2007) Impact of cattle migration from Rajasthan to Haryana state in India on transmission of tropical theileriosis. *J Vet Parasitol* 21:87–88
- Sangwan AK, Chhabra MB, Samantaray S (1986) *Theileria* infectivity of *Hyalomma* ticks in Haryana, India. *Trop Anim Health Prod* 18:149–154
- Sangwan AK, Chhabra MB, Samantaray S (1989) Relative role of male and female *Hyalomma anatolicum anatolicum* ticks in *Theileria* transmission. *Vet Parasitol* 31:83–87
- Sangwan AK, Chaudhari SS, Sangwan N, Gupta RP (1994) Seasonal distribution of *Theileria* in *Hyalomma anatolicum anatolicum* ticks in an endemic area of Haryana, India. *Trop Anim Health Prod* 26:241–246
- Sayin F, Karaer Z, Dincer S, Cakmak A, Inci A, Yukari BA, Eren H, Vatansever Z, Nalbantoglu S, Melrose TR (2003) A comparison of susceptibilities to infection of four species of *Hyalomma* ticks with *Theileria annulata*. *Vet Parasitol* 113:115–121
- Solomon ME (1951) Control of humidity with potassium hydroxide, sulphuric acid or other solutions. *Bull Entomol Res* 42:543–554
- Walker AR, McKeller SB, Bell LJ, Brown CGD (1979) Rapid quantitative assessment of *Theileria* infection in ticks. *Trop Anim Health Prod* 11:21–26
- Walker AR, Young AS, Leitch BL (1981) Assessment of *Theileria* infection in *Rhipicephalus appendiculatus* ticks collected from the field. *Z Parasitenkd* 65:63–69
- Walker AR, Latif AA, Morzaria SP, Jongejan F (1983) Natural infection rates of *Hyalomma anatolicum anatolicum* with *Theileria* in Sudan. *Res Vet Sci* 35:87–90
- Young AS, Leitch BL (1981) Epidemiology of East Coast Fever: some effects of temperature on the development of *Theileria parva* in the tick vector *Rhipicephalus appendiculatus*. *Parasitol* 83:199–211
- Young AS, Leitch BL, Staff DA, Dolan TA (1983) Identification of *Theileria* infection in living salivary glands of ticks. *Parasitology* 86:519–528
- Young AS, Leitch BL, Newson RM, Cuning MP (1986) Maintenance of *Theileria parva* infection on an endemic area of Kenya. *Parasitology* 93:9–16