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Infectivity rate and transmission potential of *Hyalomma anatolicum anatolicum* ticks for *Babesia equi* infection

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Abstract

The infectivity rate of *Babesia equi* in the salivary glands of *Hyalomma anatolicum anatolicum* was assessed. The hungry nymphs were fed on a donkey experimentally infected with *B. equi*. The engorged dropped-off nymphs were collected at different levels of parasitaemia and kept in BOD incubator. After ecdysis, the hungry adults were prefed on rabbits for different time intervals, thereafter the salivary glands were dissected out and acini were examined after methyl green pyronin (MGP) staining. A total of 134 male and 139 female ticks were dissected out. Average infected acini per tick were found to be significantly higher ($p < 0.05$) in male as compared to the female ticks. Further, maximum infected acini in both male and female ticks were found at 24 h of prefeeding on rabbits and overall infected acini per tick increased with rise in parasitaemia. The release of infected ticks on susceptible donkeys resulted in development of clinical babesiosis.

Keywords: *Babesia equi*; *Hyalomma anatolicum anatolicum*; Infectivity rate; Salivary glands; Tick; Transmission potential

1. Introduction

Equine babesiosis caused by *Babesia (Theileria) equi* and/or *Babesia caballi*, is recognized as a serious problem of major economic importance as the affected animals manifest decreased working capacity, loss of appetite, etc. (Friedhoff et al., 1990; Hailat et al., 1997). The disease condition caused by these parasites is distributed world-wide including Asian continent, Europe, Africa, South America and prevalence synchronizes with the existence of the tick-vector. Australia, United States of America, Great Britain, Germany, Switzerland, Austria and Japan are apparently free from this disease condition (de Waal, 1992). Most clinical cases are due to *B. equi* parasites and intrauterine infection to the fetus is the most serious complication of the infection (Kuttler, 1988; de Waal and van Heerden, 1994). Animals those recover from babesiosis usually remain asymptomatic carriers with positive antibody titres throughout the life time (Schein, 1988; de Waal, 1992; Ali et al., 1996).

Transmission of equine babesiosis generally falls into two categories—tick transmission and iatrogenic transmission (Knowles, 1988; de Waal and van Heerden, 1994; Gerstenberg et al., 1998). Iatrogenic spread is by contaminated hypodermic needles and

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syringes and further natural transmission will be restricted if the biological tick vectors are absent in the area. In enzootic areas, equine babesiosis is transmitted by Ixodid tick species of genera, *Hyalomma*, *Dermacentor* and *Rhipicephalus* (Schein, 1988; Ali et al., 1996), which are distributed world-wide especially in tropical and sub-tropical regions including India (Geevarghese et al., 1997). *Hyalomma* species can adapt well to dry hot and cold ecosystems and are largely distributed in Africa, south-eastern Europe and Asian regions. Twenty six species of the *Hyalomma* are world-wide distributed (Geevarghese and Dhanda, 1987a) and out of it nine species are common in Indian regions and *Hyalomma anatolicum anatolicum* is the most widely distributed species (Geevarghese and Dhanda, 1987b). *H. a. anatolicum* have been shown to transmit *B. equi* and *Theileria annulata* in equids and bovine respectively in India (Chaudhuri et al., 1969; Bhattacharylu et al., 1975). Recently, *Boophilus microplus*, which is generally a cattle tick, has also been reported to transmit *B. equi* (Mehlhorn and Schein, 1998; Guimaraes et al., 1998a,b; Ueti et al., 2003, 2005). However, the literature regarding the demonstration of *B. equi* sporozoites/sporoblasts in the acini of the salivary glands of the vector tick *H. a. anatolicum* in Asian continent is scanty. This prompted us to study the infectivity of *B. equi* in *H. a. anatolicum* ticks upon being fed on experimentally infected donkeys and transmission potential thereof to the natural host.

2. Materials and methods

2.1. Experimental animals

Seven donkeys (1–1.5 years old) were used in the present study. Their *B. equi* free status was ascertained by examining blood smears for the absence of the parasite for three consecutive days and serologically by Dot-ELISA (Kumar et al., 1997). All the donkeys were maintained in tick-free animal shed of All India Coordinated Research Project on Blood Protista (AICRP), College of Veterinary Sciences, Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar. However, at the time of tick feeding, they were transferred to an ordinary animal shed. Hisar strain of *B. equi* was maintained in a spleen-intact donkey, and its blood was inoculated to a naïve donkey so as to produce experimental infection. Six donkeys were divided into two groups: group I comprised of four donkeys, which were used to study the transmission potential of adult *B. equi* infected *H. a. anatolicum* ticks. Remaining two donkeys in group II were used as non-infected controls.

2.2. Babesia equi infected ticks

Engorged dropped off adult female *H. a. anatolicum* ticks (identified as per key defined by Kaiser and Hoogstraal, 1964) were obtained from experimental calves maintained for rearing tick colonies, incubated at 28 °C and 85% relative humidity (RH) for egg laying and hatching in a BOD incubator. Hungry larvae were released on ears of rabbits (New Zealand White) and collected upon engorging, kept at 28 °C and 85% RH. Hungry uninfected nymphs were stored at 18 °C and 85% RH.

For raising *B. equi* infected ticks, about 1500 hungry uninfected nymphs (as above) were released on both the ears of an experimental donkey which previously had been got infected with *B. equi* parasite by inoculating blood collected from a carrier donkey (as above) and thereafter splenectomised. Engorged dropped-off nymphs were collected and percent parasitaemia at the time of collection was recorded viz. up to 5, 5–20 and 20–55%. Parasitaemia in peripheral circulation of experimental infected donkey was determined by counting at least about 2000 erythrocytes under the microscope per stained smear. Engorged nymphs were incubated at 28 °C and 85% RH in a BOD incubator for moulting to adult stage. Male and female hungry adult ticks were separated and stored at 18 °C and 85% RH.

These ticks were pre-fed on rabbits so as to study the effect of pre-feeding on maturation of *B. equi* sporozoites in the salivary glands, if any. The salivary glands of pre-fed ticks were dissected out (Blewett and Branagan, 1973) and stained with methyl green pyronin (MGP) following the procedure described by Walker et al. (1979) adopting some modifications. Briefly, salivary glands were fixed in absolute ethanol for 10 min and subsequently immersed in MGP stain for different time duration i.e. 30, 45 or 60 min. Thereafter the glands were washed with absolute ethanol for 2 min and dehydrated in absolute ethanol for 5 min and cleared in xylol for 2 min. The glands were teased out on a glass slide, cover-slipped with DPX mounting medium, dried and examined under light microscope. All animal experiments were done as per ethically approved methods and procedures.

2.3. Infectivity rate and size of infected acini

Morphology of normal salivary gland acini was established after staining with MGP and compared with