Infection Rates of *Theileria sergenti* in *Haemaphysalis longicornis* Ticks Collected from the Field in Japan

Tsugihiko KAMIO, Yasuhiro ITO¹⁾, Kozo FUJISAKI, and Tetsuro MINAMI

First Research Division, National Institute of Animal Health, Tsukuba, Ibaraki 305 and ¹⁾National Grassland Research Institute, Nishinasuno, Tochigi 329-27, Japan

(Received 22 June 1989/Accepted 22 September 1989)

ABSTRACT. For a period of one year *Haemaphysalis longicornis* ticks were collected in a pasture from vegetation by dragging flannel cloth and from soil samples at monthly intervals. Nymphal ticks were assessed for *Theileria* infection. Salivary glands were stained with methylgreen-pyronin and examined for parasite masses. Nymphal *H. longicornis* infected with *Theileria sergenti* were found in all samples during 12 months including the winter. After shortening the prefed period on rabbits to 24 hours, the parasite masses could be detected in the salivary glands of nymphs collected in the grazing season, from May to October, while no parasite masses were detected in other season. It was suggested that the environmental factors in the grazing season might influence on the maturation of parasites in the salivary glands of ticks.—**KEY WORDS:** *Haemaphysalis longicornis*, infection rate, *Theileria sergenti*.

Jpn. J. Vet. Sci. 52(1): 43–48, 1990

Haemaphysalis longicornis has been recognized as the most important vector of *Theileria sergenti* in Japan [4]. The natural infection rate of ticks with *T. sergenti* is an important parameter in the epidemiology of bovine theileriosis. There is, however, little information on the seasonal assessment of *Theileria* infection *H. longicornis* in a pasture. Recently Ito *et al.* [3] discovered that all the developmental stages of *H. longicornis*, except eggs, overwintered in the soil of pasture. Fujisaki *et al.* [1] demonstrated that *T. sergenti* was found in the salivary glands of nymphal *H. longicornis* overwintering in a pasture and also they suggested that the infected ticks, after overwintering, might play an important role in spring outbreaks of bovine theileriosis. In addition, there is considerable discussion on the valid name of the organism designated as *T. sergenti* in Japan [9]. We used tentatively the well known name *T. sergenti* in this paper.

In the present study, we examined nymphal *H. longicornis* collected from pasture during one year for the presence of theilerial protozoa, in order to study the epidemiolo-

gy of bovine theileriosis in Japan.

MATERIALS AND METHODS

Location: Collection of ticks in the field were made at the Ikeda ranch located at the southern foot of the Nasu Mountains, about 500–600 m above sea level in Tochigi Prefecture, Japan. On this pasture Japanese Black cows were mainly grazed from May to October and Azuma-nezasa, *Pleioblastus chino* Makino, was the dominant pasture plant. Cattle grazing on this pasture showed no clinical signs of *T. sergenti* infection but a high prevalence of intraerythrocytic forms, piroplasms, of *T. sergenti* were found.

Tick collection and processing: During the periods from October 1984 to March 1985, unfed ticks overwintering in the soil were collected monthly from the soil samples, which were dug out of the surface of the ground, about 15 cm in square and 4–5 cm in depth including litter [3]. Unfed ticks were also collected by dragging, 10 m each drag, with a piece (1 × 0.7 m) of white cotton-flannel cloth on the vegetation on nine

occasions during five months from April to October 1984. The ticks were also collected by rubbing with the cloth on the vegetation supplementarily. The identity of the ticks was confirmed as *H. longicornis*. Collected ticks were stored at 25°C constant temperature and saturated humidity in continual darkness for 10 to 30 days after collection. Only nymphal ticks were used in subsequent examinations.

Salivary glands examination: For *Theileria* detection nymphal ticks were assessed according to Fujisaki *et al.* [1] and Kamio *et al.* [6]. Ticks, 50 to 60 ticks from each month samples, were applied to the ears of immature commercial domestic rabbits to activate parasites in salivary glands [5]. And then, 10 ticks of them were removed as the partially engorged state, 6, 12 and 24 hours after attachment. Alternatively, 20 to 25 ticks of them were removed 48 hours after attachment. Afterwards, their salivary glands were dissected out in sterile saline under dissecting microscope and examined for the presence of theilerial protozoa after staining with methylgreen-pyronin [10].

Haematological examination of cattle: The haematological examination of cattle grazed in a pasture were carried out monthly from May to October. Total erythrocyte and leucocyte counts, packed cell volume (PCV), haemoglobin volume (cyanmethaemoglobin method), mean corpuscular volume were determined by the haemoglobin meter (Coulter Electronics, Inc., U.S.A.). Blood smear were taken from cattle, fixed in methanol, stained with Giemsa stain for *T. sergenti* piroplasms. The parasitaemia in blood smears of cattle were recorded as the percentage of parasitized erythrocytes in 10,000 erythrocytes. Particular attention was given to their PCV, which was an important parameter in occurrence of anaemia, and also piroplasm parasitaemia.

RESULTS

Seasonal occurrence of ticks: Fig. 1 summarizes the seasonal occurrence of three stages of *H. longicornis* collected from vegetation by dragging flannel cloth and from soil samples. In April, overwintering ticks occurred on the litter and plants and they were partly activated by being breathed in, although many ticks still remained on ground. The number of collected ticks and the stage composition changed markedly during the grazing season, mid-May to October. The larvae were most abundant on grasses in June and early October and they appeared to stay on the plant up to late October. Many nymphs and adults were collected from vegetation in June and July. During this period, many ticks of three stages showing various states of engorgement attached on cattle.

Ticks were collected from the soil samples from October to March of the following year. The population of ticks overwintering in the soil consisted of larvae, nymphs, and adults. The larval population was so high only in limited samples, though they occupied in highest numbers in each collecting date. Nymphs were found in most of the samples most frequently and adults were in the lowest numbers.

Fig. 1. Seasonal occurrence of three stages of *Haemaphysalis longicornis* collected from vegetation and soil samples. $\Delta \cdots \Delta$ Adult, $\bullet \cdots \bullet$ Nymph, $\circ \cdots \circ$ Larva.

Theileria in salivary glands: No theilerial parasite masses were detected in the salivary glands of nymphal stages of *H. longicornis* after 6 or 12 hours feeding on rabbits. The number of ticks examined and the numbers of infected ticks in nymphal samples after 24 hours feeding on rabbits are shown in Fig. 2. The parasite masses were detected in nymphal ticks collected from May to October, except September when ticks were not examined. Results on the numbers of infected ticks in nymphal samples after 48 hours feeding are summarized in Fig. 3. The parasitized ticks were observed from all samples of 12 months.

Mean number of infected acini per parasitized ticks was about 3 infected acini. However, some of the ticks were more heavily infected, e.g., the highest count of 96 infected acini in one tick was found in the September collection and 37, 58 and 35 acini in one tick in January, April and October, respectively.

Haematological examination of cattle: No clinical and haematological signs of theileriosis were observed in 30 Japanese Black cows examined during the grazing season. Twenty-three cows had piroplasms in their erythrocytes at low parasitaemia by blood smear examination in May. Fig. 4 shows the changes in PCV level and parasitaemia in 7 cows which were demonstrated to be free from blood parasites by blood smear examination in May. Piroplasms were detected in blood smear in all cows in June, one month after grazing. After the appearance of parasitaemia, a slight rise of mean parasitaemia observed, but the maximum level of parasitaemia showed in one cow was below 0.03%. PCV level observed in cattle during 6 months was constantly normal level.

DISCUSSION

In the present study, a seasonal survey of

Fig. 2. Detection of the numbers of infected ticks in nymphal *Haemaphysalis longicornis* after feeding on rabbits for 24 hours. ND: Not done.

Fig. 3. Detection of the numbers of infected ticks in nymphal *Haemaphysalis longicornis* after feeding on rabbits for 48 hours. ND: Not done.

Fig. 4. Changes in packed cell volume (PCV) level and parasitaemia in 7 cows in the grazing season. ○: Mean number of the whole values in 7 cows.

the infection of *H. longicornis* with *T. sergenti* on the pasture was carried out. The protozoan masses were detected in the salivary glands of nymphs collected in all months throughout one year. Therefore, the overwintering of *T. sergenti* infection in *H. longicornis* in the soil of pasture demonstrated by Fujisaki *et al.* [1] was confirmed in this study.

The infection rate of ticks examined and the stage composition collected in May indicated that the cattle were subjected to a heavy challenge of nymphal ticks responsible for spring outbreaks of bovine theileriosis. High infections were observed in some experimentally infected ticks when larvae were fed on calves with high levels of piroplasm parasitaemias [7, 11]. However, the piroplasm parasitaemias of cattle examined were at low levels during the grazing period. Young *et al.* [16] noted that important epidemiological features were the relatively low infection rates and levels in naturally infected ticks and most of these field ticks had only one acinus infected. This feature was also observed in ticks examined in our study. Therefore, in these endemic areas, ticks would usually imbibe the minimal possible dose of infective to develop infection in their salivary glands. This suggested that the natural conditions in the field, for examples, temperature, humidity or a certain diurnal temperature rhythm had a important effect on the resultant development of parasites in the pre-moult and post-moult larval ticks which had fed on infected cattle [13].

Fujisaki and Kamio [2] showed that the protozoan masses in the salivary glands of nymphs 105 days post-moulting, which were incubated at 25°C, could be detected after less than 1 hour feeding on rabbits but they were not detectable in nymphs 30 days post-moulting even after 24 hours attachment to the host. Thus, it was appeared that temperature, tick age and the other environ-

mental factors on the grazing season might be influenced on the development of *T. sergenti* in the salivary glands of *H. longicornis*. These factors could explain why the infected acini from ticks collected during grazing season were detected despite of shortening the prefed period when no parasite masses were detected during the other season. Young and Leitch [14] reported that certain alternating warm and cold periods during the pre-moulting period of *T. parva* infected nymphal or larval ticks, *Rhipicephalus appendiculatus*, could be beneficial for the production of high infections in adults and nymphal ticks.

Although Fujisaki and Kamio [2] observed no parasite masses in salivary glands of ticks incubated at 35°C for 15 days without blood meal stimulus, Samish [8] reported that a temperature of 37°C for several days stimulated the non-infective parasites of *T. annulata* in the salivary glands of unfed *Hyalomma excavatum* ticks to become infective without the previous stimulation of feeding. He discussed that failure to prevent theileriosis in hot climates by dipping cattle in acaricides every 3 days or even daily might possible be explained by the rapid appearance of infective parasites in ticks exposed to high ambient temperatures, even before they had a blood meal. It was considered that *Theileria* parasites appear to be adapted for development in ticks under natural climatic conditions in the field.

In this study, 23 cows had piroplasms in their erythrocytes at low parasitaemia by blood smear examination in May. It would appear that these cows could become carriers of *T. sergenti*. It is unknown whether or not *T. sergenti* can persist in cattle and infect ticks over long period. The duration of *Theileria* parasite within the tick has an important bearing on control measures against theileriosis.

Young *et al.* [15] stated an accurate period

for parasite and tick survival under simulated natural conditions. Furthermore, Young *et al.* [16] demonstrated the first conclusive proof that naturally infected cattle have a prolonged carrier state of *T. parva parva*. It would appear important to study how *T. sergenti* infection is maintained in the field. The actual mechanism by which the natural carrier state of *T. sergenti* is maintained in cattle and ticks should be determined. Therefore, it is essential to repeat this study in other areas of Japan.

Simple staining method to examine ticks were in use to study *T. parva* and *T. annulata* infection in field ticks [11, 12]. The methylgreen-pyronin staining method, which was recently applied successfully to *H. longicornis* infected with *T. sergenti* [1, 6], has played a valuable role in this study. However, for detecting and assessing *Theileria* at very low field infection rates and/or in very low populations of ticks, sporozoite concentration methods may be useful [11]. Furthermore, for field surveys to study the distribution and frequency of piroplasms infecting ticks, which requires the examination for large samples of ticks, a simplified and rapid detection system appear to be essential.

ACKNOWLEDGEMENTS. We are grateful to Dr. A. S. Young (Veterinary Research Department, Kenya Agricultural Research Institute) for reviewing our manuscript. We also wish to thank Dr. T. Matsumura and the staff of the National Grassland Research Institute for their help in carrying out the experiment.

REFERENCES

1. Fujisaki, K., Ito, Y., Kamio, T., and Kitaoka, S. 1985. The presence of *Theileria sergenti* in *Haemaphysalis longicornis* overwintering in pasture in Japan. *Ann. Trop. Med. Parasitol.* 79: 519-534.
2. Fujisaki, K. and 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.
3. Ito, Y., Watanabe, N., and Matsumura, T. 1983. Overwintering of *Haemaphysalis longicornis* (Acari: Ixodidae) at a pasture, Nasu plateau, Japan. *Bull. Natl. Grass. Res. Inst.* 24: 110-118.
4. Ishihara, T. 1968. Bovine piroplasmiasis in Japan. *Jpn. Agr. Res. Q.* 3: 23-31.
5. Kamio, T., Fujisaki, K., and Minami, T. 1987. The improvement of "ear bag" method for tick infestation. *Proc. Jpn. Assoc. Acarol.* 14: 1-4.
6. Kamio, T., Fujisaki, K., and Minami, T. 1989. Correlation between the infection rate of vector tick, *Haemaphysalis longicornis* and the parasitaemia of cattle infected with *Theileria sergenti*. *Ann. Trop. Med. Parasitol.* 83: 77-83.
7. Purnell, R. E., Ledger, M. A., Omwoyo, P. L., Payne, R. C., and Peirce, M. A. 1974. *Theileria parva*: Variation in the infection rate of the vector tick, *Rhipicephalus appendiculatus*. *Int. J. Parasitol.* 4: 513-517.
8. Samish, M. 1977. Infective *Theileria annulata* in the tick without a blood meal stimulus. *Nature (Lond.)* 270: 51-52.
9. Uilenberg, G. 1981. Theilerial species of domestic livestock. pp. 4-37. In: *Advances in the control of theileriosis*. (Irvin, A. D., Cunningham, M. P., and Young, A. S. eds.), Martinus Nijhoff, The Hague.
10. Walker, A. R., McKellar, S. B., Bell, L. J., and Brown, C. G. D. 1979. Rapid quantitative assessment of *Theileria* infection in ticks. *Trop. Anim. Health Prod.* 11: 21-26.
11. Walker, A. R., Young, A. S., and Leitch, B. L. 1981. Assessment of *Theileria* infections in *Rhipicephalus appendiculatus* ticks collected from field. *Z. Parasitenkd.* 65: 63-69.
12. Walker, A. R., Latif, A. A., Morzaria, S. P., and Jongejans, F. 1983. Natural infection rate of *Hyalomma anatolicum anatolicum* with theileria in Sudan. *Res. Vet. Sci.* 35: 87-90.
13. Young, A. S. and Leitch, B. L. 1981. Production of *Rhipicephalus appendiculatus* with high infections of *Theileria parva*. *J. Parasitol.* 67: 751-752.
14. Young, A. S. and Leitch, B. L. 1981. Epidemiology of East Coast fever: some effects of temperature on the development of *Theileria parva* in the tick vector, *Rhipicephalus appendiculatus*. *Parasitology* 83: 199-211.
15. Young, A. S., Leitch, B. L., Dolan, T. T., Newson, R. M., Ngumi, P. N., and Omwoyo, P. L. 1983. Transmission of *Theileria parva* by a production on *Rhipicephalus appendiculatus* under simulated natural conditions. *Parasitology* 86: 255-267.
16. Young, A. S., Leitch, B. L., Newson, R. M., and Cunningham, M. P. 1986. Maintenance of *Theileria parva parva* infection on an endemic area of Kenya. *Parasitology* 93: 9-16.

要 約

野外より採集された *Haemaphysalis longicornis* における *Theileria sergenti* 感染状況：神尾次彦・伊戸泰博¹⁾・藤崎幸蔵・南 哲郎（農林水産省家畜衛生試験場，¹⁾ 農林水産省草地試験場）——栃木県下の1牧野において *Haemaphysalis longicornis* の発生活長を周年調査し，未吸血若ダニ唾液腺内の *Theileria sergenti* の感染状況を調べた．マダニの採集はフランネルを用いたハタズリ法及び土壌サンプル採集法を併用して行い，*T. sergenti* の感染状況は，若ダニを家兔に吸血させて半飽血状態とした後，唾液腺を摘出し，メチルグリーン・ピロニン染色することによって行った．その結果，本牧野に生息する若ダニは，冬期を含んだ1年を通じて *T. sergenti* を保有していることが明らかとなった．放牧期間の5月から10月にかけて採集された若ダニでは，家兔吸血24時間目に唾液腺内の原虫が検出されたのに対し，他の時期に採集された若ダニでは検出されなかった．この結果から，放牧期間中の環境要因が若ダニ唾液腺内の原虫成熟に何らかの影響を及ぼしていることが示唆された．