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Reversion of *Theileria sergenti* merozoite to schizont

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Theileria sergenti 분열소체(merozoite)의 분열전체(schizont)로의 복귀

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초 록 : *Theileria* spp.의 생활환에 대해서는 여러종류의 책을 통해서 잘 소개되어 있다. 그중 *Theileria* spp.의 분열소체 즉, merozoite는 주로 숙주의 적혈구내에 존재하는데 진드기에 의해 흡혈되지 않으면 더이상 발육하지 못하고 생을 마감한다고 여겨 왔다. 그러나 적혈구내 merozoite가 임파구에 다시 들어가 schizont로 복귀하여 분열·증식된다는 가설은 아직까지 증명된 바 없다. 본 실험은 *T sergenti* merozoite의 schizont로의 복귀를 입증하고자 수행되었다. *T sergenti* 에 감염되지 않은 3개월령의 송아지를 비장적출시킨 후 *T sergenti* merozoite에 감염된 순수적혈구를 인공감염시켰다. 인공감염후 경시적으로 혈액과 임파액을 채취하여 적혈구내 *T sergenti* 감염을 조사하고 백혈구 감별혈구를 계산하였으며 임파구내 schizont 출현을 관찰하여 다음과 같은 결과를 얻었다.

1. 혈구내원충 감염율(parasitemia : PE)은 인공감염후 28일째 최고치인 10.5%를 보였으며 그후 5% 이내의 수준을 유지하다가 70일째 다시 8.5%의 상승점을 보였다.

2. 백혈구 감별혈구계산에서는 감염초기에는 호중구가 주종을 이루다가 감염후 19일을 기점으로 임파구(60~80%)가 급격히 증가하여 실험종료 때까지 유지되었다.

3. 인공감염후 19~23일, 59~63일 사이에 말초혈액내 임파구에서 분열·증식하고 있는 schizont를 관찰할 수 있었다.

4. 인공감염후 7일부터 림프액내 임파구의 크기가 커지면서 blast-formation이 진행되었으며 실험종료때까지 유지되었다.

이상의 결과로 보아 적혈구내 merozoite가 임파구에 다시 들어가 schizont로 복귀하여 분열·증식함을 입증하여 기존의 *T sergenti* 생활사는 수정되어야 된다고 사료된다.

Key words : *Theileria sergenti*, development, schizont, merozoite, lymphocyte, lymph node.

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Introduction

The diseases called theileriosis and babesiosis cause fever and anemia and lead to huge economic losses through the world. Over 80 percentage of 1200 million cattle in the world are at risk of tick infestation and tick-born diseases. The piroplasms containing mainly *Theilera* spp. and *Babesia* spp. are highly pathogenic to cattle, sheep, goats and sometimes to man. The theileriosis are still the most important disease in Korea especially in dairy cattle in spite of the long period of effort of farmers and government to control this disease.

The parasites located in the blood cells of their vertebrate hosts are transmitted by the broad spectrum of the ixodid tick species[1,2]. Their life cycles had been incompletely known because these parasites are very small and morphologically undefinable by light microscopy[3]. Thus only asexual reproduction within the salivary glands of ticks and within the blood cells of vertebrates could be identified. The clear identification of a typical sporozoan life cycle for piroplasms, however, comprising the three typical phases: schizogony, gametogony and sporogony were carried out with the laboratory transmission experiments and *in vitro* culture[3].

The life cycle of *T parva* causing east coast fever in Africa have been investigated by many researchers and it has been recognized as the life cycle of *Theileria* spp. in general [4,5].

In experiments about *Theileria sergenti* (*T sergenti*), the development of *T sergenti* in the the gut and salivary gland of vector tick has been reported[6-8] and Sasaki[9] reported that the environmental temperature could affect the sucking ability and the maturation of *T sergenti* in salivary glands of ticks. Though the development of *T sergenti* schizonts in the lymph node of cattle has been reported recently by Sato [10], most of experiments has been carried out in ticks.

The development of *T sergenti*, the main causative agent of piroplasms in cattle, has been investigated mostly about the morphology at certain stage[11-15].

The life cycle of *T sergenti* is well known as the merozoite in the erythrocyte of cattle is taken by ticks and develops to sporozoite in the salivary gland of ticks and enter the host when ticks suck the host again. After the parasite invade the host, it develops schizonts in lymphocytes and merozoite in erythrocytes. The merozoite ends its life unless it enter the gut of tick when the tick suck the blood. There are no reports available on the possibility that the merozoite of *T sergenti* in the erythrocyte could invade lymphocyte and develop to schizont again called reversion.

In this study, we describe the development of the schizont in the peripheral lymphocyte at interval after *T sergenti* infected bovine-erythrocyte was artificially inoculated to the splenectomized calf. The result suggest that the merozoite of *Theileria* spp. could be reverted to the schizont during the development.

Materials and Methods

Animal : A Korean native calf aged 3 months was used in this experiment. No parasites were detected in Giemsa-stained blood smear and PCR before use. The calf used in this experiment was splenectomized.

Parasite : Sunghwan stock of *T sergenti* isolated from Sunghwan, Korea was used in this experiment. This stock had been maintained in our institute by passage through splenctomized calves.

Artificial inoculation of parasite : After 30ml of whole bood was collected from the calf infected by *T sergenti*, Sunghwan stock, erythrocytes were taken by washing and centrifuged with phosphate buffered saline(PBS, pH 7.4) three times. To confirm the existence of erythrocytes without lymphocyte, blood smear was stained by Giemsa's solution and examined the boold cell under light microscope. 10ml of pure and fresh erythrocytes were inoculated to splenectomized calves intravenously.

Hematocrit(PCV), Parasitemia(PE), and Leukocyte Differential Count : Peripheral blood samples were collected from the calf immediately before and at 2~3 days intervals after artificial inoculation of *T sergenti*-infected RBC. PCV was measured by conventional methods. Parasitemia (PPE: percentage of parasitised erythrocytes) was as-

sessed by examination of Giemsa-stained blood smears. Leukocyte differential count(percentage) was measured by counting eosinophils, neutrophils, basophils, lymphocytes and monocytes in total 200 leukocytes.

Isolation and staining of peripheral lymphocytes : For detecting the schizont in peripheral lymphocyte after inoculation, buffy coat of whole blood centrifuged was isolated and stained. 1ml of ACD solution was mixed with 9ml of whole blood and centrifuged at 700g for 20 mins. Buffy coat was isolated and disolved in PBS(pH 7.4). The disolved materials was loaded on Histopaque Ficoll(Sigma Co.) and centrifuged at 700g for 40 mins. After the band was isolated from the percoll column, 4ml of RBC lysing buffer(Sigma Co.) was added and agitated for 40 secs following adding 2ml of PBS(pH 7.4) and centrifuging at 700g for 5 mins. This procedure was repeated again to remove erythrocytes. After washing the lymphocyte with the centrifugation at 700g for 5 mins twice, the sediment was smeared on the slide and Giemsa-stained for microsopic observation.

The drainage lymph node : To observe any changes in lymph node during the infection, lymphoid fluid was collected from the prescapular lymph node just before and at 2~3 days intervals after artificial inoculation from the calf. Collected lymphoid fluid was centrifuged at 700g for 10 mins and the sediments was smeared on the slide and Giemsa-stained for microscopic observation.

Results

Hematocrit : It was decreased gradually until 20 days after artificial inoculation and decreased sharply between 21 days and 26 days. The lowest level of hematocrit was shown on 36~38 days as 17% after artificial infection. And the hematocrit level was kept 20~30% and could not come over the 30% till the end of experiment (Fig 1).

Merozoite in erythrocytes(PE) : Artificial infection was confirmed as 0.07% of parasitemia(PE: percentage of parasitised erythrocytes) on 7 days after inoculation. The highest level of PE was detected as 10.5% on 28 days after inoculation. After that, PE was continued to be shown as 5~

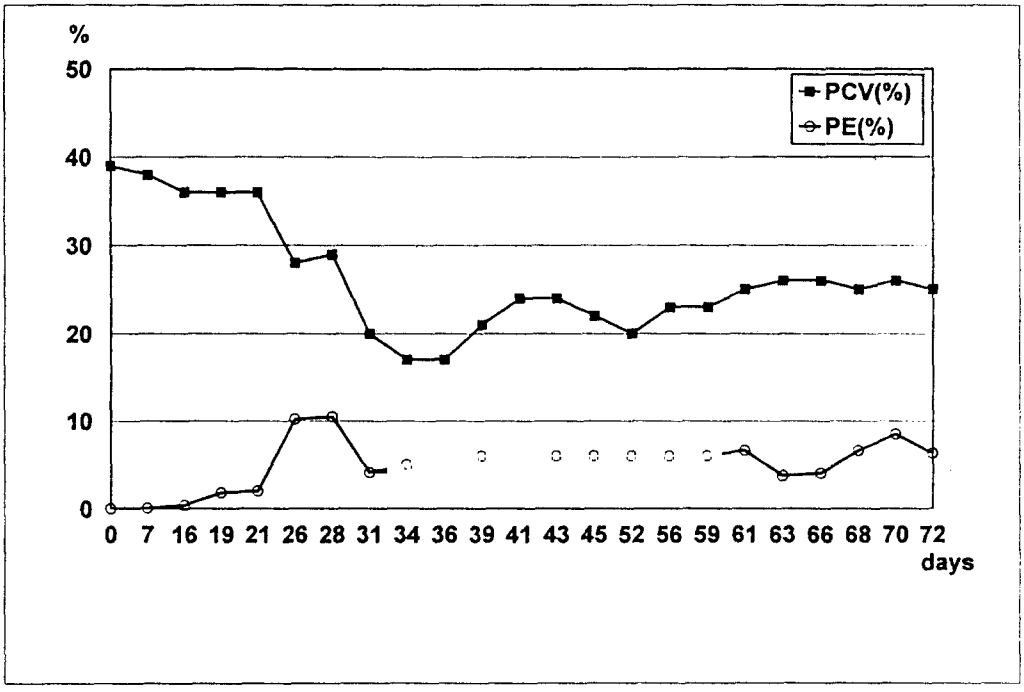


Fig 1. Changes of PCV and PE during the artificial infection.

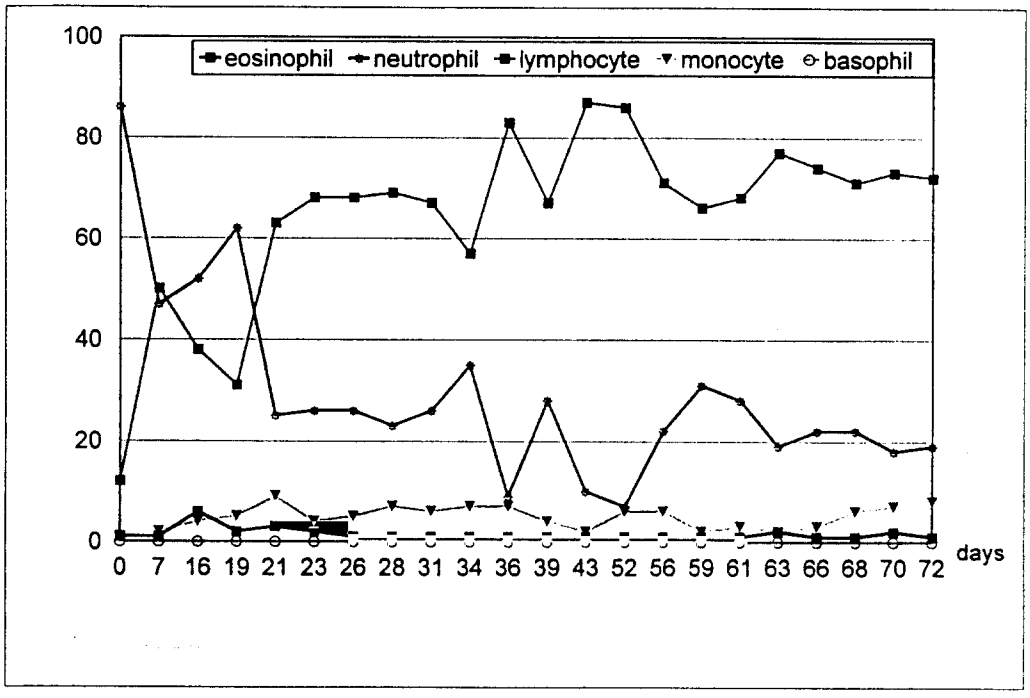


Fig 2. Leukocytes differential counting during artificial infection.

7% and 7.5% on 70 days.

Leukocytes differential counting : The number of neutrophils have been much more than the number of lymphocytes from 0 day to 19 days after artificial infection. From 21 days, The lymphocytes were increased dramatically otherwise the neutrophils were decreased markedly and this situation was kept to the end of experiment (Fig 2).

Schizont in lymphocyte : The schizonts of *T sergenti* were observed in the peripheral lymphocyte with the period of 19~23 days and 61~63 days after artificial infection (Fig 3, 4).

Blast-formation in lymph node : Lymphoid cells were become to be enlarged and to be blast-formed gradually from 7 days after artificial infection. The Blast-formation was advanced according to the progress of the infection (Fig 5, 6, 7).

Discussion

It is the general understanding that the merozoite of *Theileria* spp. in the erythrocyte ends its life unless it enter the gut of ticks when the ticks suck the blood of cattle. However, Barnett[16] raised the question of whether *T parva* can be transmitted by blood inoculation and also whether such inoculated piroplams can revert to schizont.

In this study, We have carried out to investigate the development of the schizont in the peripheral lymphocyte after *T sergenti* infected bovine-erythrocyte was artificially inoculated to the splenectomized calf to prove the Barnett's questions.

In Korea, a lot of dairy cattle were imported from foreign countries from the late of 1960s. The imported cattle have no resistance to *T sergenti* genetically and provoked clinical symptoms of theileriosis, fever, anemia, weight-loss, sometimes death eventhough the domestic native cattle seldomly showed clinical sign. This disease has been emerged the big barrier to the development of the dairy cattle industry.

There have been many reports of experiments about *T sergenti* as diagnostic methods, surveys on the prevalance, fine structure of the developmental stage and control methods.

But the recognition of the life cyle of *T sergenti* is still depend on the life cycle of *T parva* .

DeMartini and Moulton[17] represented that the change in the drainage lymph nodes attributed to *T parva* infection are utmost enlargement of the paracortical zone due to infiltration and proliferation of parasitized lymphocyte, often in mitosis, and there are a lot of lymphoblasts in the sinuses and medullary cords. In this study, we could abserve many lymphoblasts in the drainage lymph node from the *T sergenti* -infected splenectomized calf. It shows that this study agree with the DeMartini and Moulton's results. It is the typical recognition of blood feature in cattle infected by *Theileria* spp. that the number of neutrophils were decreased from the days 23 after infection otherwise the number of lymphocytes were sharply increased from the days 21 after infection[18].

Also Sato *et al*[13] observed that a lot of huge cells containing granules in the cytoplasm in the local drainage nodes such the parotid and retropharyngeal nodes, livers and spleens and confirmed that the granules were exo-erythrocytic forms of *T sergenti* because they showed a specific immunohistichemical reaction against anti-*T sergenti* antibody. But we could not observe any huge cells including granules in this experiment.

A single schizont of *T sergenti* grows into two forms. It appears to be a macroschizont during the phase of nuclear division and a microschizont with rosette-like appearance during merozoite formation[11]. According to this definition, The schizonts of *T sergenti* observed in this study were considered the microschizont becuse they produced merozoite soon. The schizont of *T parva* and *T annulate* are formed in lymphocyte or monocytes and mature only in lymphoid cells[19].

Schizonts of *T sergenti* were observed in lymphocyte between 19~23 days and 61~63 days after inoculation in this study. Although there is not yet obtained enough evidence of the reversion to the schizont, we consider that the merozoite of *T sergenti* in erythrocyte would invade the lymphocyte and revert to the schizont and produce the merozoite again. It is thought that electron microscopic observation is need to confirm this new concept about the development of *T sergenti* life cycle in near future.

Legends for figures

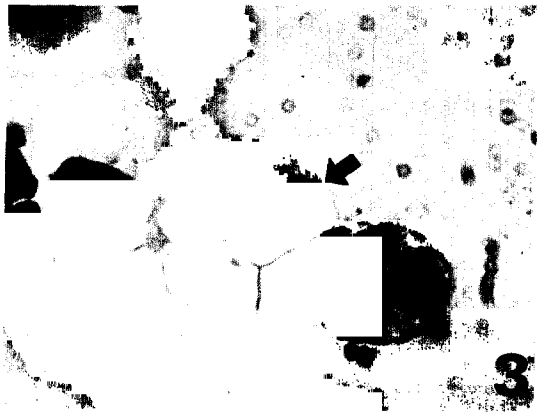
Fig 3. *T sergenti* schizont(arrow) in lymphocyte on 23 days after artificial infection.

Fig 4. *T sergenti* achizont(arrow) in lymphocyte on 62 days after artificial infection.

Fig 5. Normal lymphoid cells in lymph node.

Fig 6. On 7 days after artificial infection, Lymphoid cells have been enlarged and blastoid(arrow).

Fig 7. Blast-formation of the lymphoid cells on 20 days after artificial infection(arrow).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Referances

1. Riek RF. The life cycle of *Babesia argentina* (Lignieres, 1903) in the tick vector *Boophilus microplus* (Canestrin). *Australian J of Agri Res*, 17:247-254, 1996.
2. Friedhoff KT, Smith RD. *Babesiosis Academic Press*. p267-321, 1981.
3. Mehlhorn H. The piroplasms: lifecycle and sexual stages. *Adv in Parasitol*, 23:37-103, 1984.
4. Koch R. Beitrage zur Entwicklungsgeschichte der Piroplasmen. *Zeitschrift fur Hygiene und Infektionskrankheiten*, 54:1-9, 1996.
5. Schein E, Warnecke M, Kirmse P. Development of *Theileria parva* in the gut of *Rhipicephalus appendiculatus*. *Parasitology*, 75:309-316, 1997.
6. Higuchi S. Development of *Theileria sergenti* in the midgut of the larval tick *Haemaphysalis longicornis*. *Kitasato Arch Exp Med*, 60:71-77, 1987.
7. Higuchi S. The development of *Theileria sergenti* in the salivary glands of the tick, *Haemaphysalis longicornis*. *Nippon Juigaku Zasshi*, 48:801-807, 1986.
8. Higuchi S. Development of *Theileria sergenti* in the ovary and eggs of the tick, *Haemaphysalis longicornis*. *Kitasato Arch Exp Med*, 58:117-25, 1985.
9. Sasaki N. Maturation of *Theileria sergenti* in salivary glands of *Haemaphysalis longicornis* induced by temperature stimulation. *Nippon Juigaku Zasshi*, 52:495-501, 1990.
10. Sato M. Development of *Theileria sergenti* schizonts in the lymph node of experimentally infected cattle. *J Vet Med Sci*, 56:715-722, 1994.
11. Shimizu S, Suzuki K, Nakamura K. *et al*. Isolation of *Theileria sergenti* piroplasms from infected erythrocytes and development of enzyme-linked immunosorbent assay for serodiagnosis of *T sergenti* infection. *Res Vet Sci*, 45:206-212, 1988.
12. Kawazu S, Sato M, Tanaka S, *et al*. Histological staining of *Theileria sergenti* schizonts. *J Vet Med Sci*, 53: 743-745, 1991.
13. Sato M, Kamio T, Kawazu S, *et al*. Histological observations on the schizonts in cattle infected with Japanese *Theileria sergenti*. *J Vet Med Sci*, 55:571-574, 1993.
14. Sato M, Kamio T, Tanaka S, *et al.* Development of *Theileria sergenti* schizonts in the lymph node of experimentally infected cattle. *J Vet Med Sci*, 56:715-722, 1994.
15. Lee SL, Lee JM, Kwon OD, *et al*. Detection of *Theileria sergenti* schizonts in lymphpoid cell of lymph node in Korean cattle. *Korean J Vet Res*, 37:189-194, 1997.
16. Barnett SF. Parasite protozoa vol. IV Academic Press, New York. p77-113, 1977.
17. DeMartini JC, Moulton JE. Response of the bovine lymphatic system to infection by *Theileria sergentie*. I. Histology and ultrastructure of lymph nodes in experimentally-infected calves. *J Comp Pathol*, 83:281-298, 1973.
18. Irvin AD, Morrisen W1. Immune responses in Parasitic Infection vol III, CRC Press, Florida. pp223-274, 1987.
19. Uilenberg G, Perie NM, Spanjer AAM, *et al. Theileria orientalis*, a cosmopolitan blood parasite of cattle : demonstration of the schizont stage. *Res Vet Sci*, 38:352-357, 1985.