

Prevalence of *Theileria sergenti* infection in Korean native cattle by polymerase chain reaction

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Abstract: This study was performed to investigate the prevalence of theileriosis and to compare the prevalence of this disease in Korean native cattle reared under different environmental conditions, namely, in a grazing area and a non-grazing area by polymerase chain reaction. Three hundred and one Korean native cattle (276 cows and 25 bulls) that had not received prior treatment or been vaccinated to prevent theileriosis were examined by PCR for *Theileria sergenti* infection from 2001 to 2002. In our study, the parasitemia range in *T. sergenti*-positive cattle by microscopy were from 0.1 to 3% (mean 0.8%). In terms of mean prevalence, 204 of the 301 Korean native cattle (67.8%) were positive reaction by PCR. Our results also revealed that the infection rate among cows (70.3%) was significantly higher than that among bulls (40.0%) ($p < 0.01$). *T. sergenti* infection among the over 3 year-old-group (75%) had a significant higher prevalence than that among the less than 3 year-old-group (61.8%) ($p < 0.05$). Our data also showed that grazing areas (76.1%) had the significant higher prevalence than non-grazing areas (51%) ($p < 0.001$). In conclusion, this study demonstrates that the prevalence of *T. sergenti* infection is high and that its prevalence in grazing cattle is higher than that in non-grazing cattle. Therefore, life-long treatment and the development of an optimal vaccine are needed to reduce the numbers of bovine theileriosis in both grazing and non-grazing areas.

Key words: theileriosis, prevalence, polymerase chain reaction, Korean native cattle

INTRODUCTION

Theileriosis, a tick-borne hemoprotozoan disease, is one of the more important diseases of grazing cattle in Korea (Kim et al., 1993; Chae et al., 1996; Choi et al., 1997). The main symptoms shown by infected cattle are fever and chronic anemia, due to intraerythrocytic parasitism by piroplasms (Chae et al., 1996; Shiono et al., 2001).

Theileria sergenti has been found to be associated with the infection of cattle in East Asia (Kubota et al., 1996; Choi et al., 1997), Southern Asia (Wang et al., 1998; Chansiri and Sarataphan, 2002) and Europe (Savini et al., 1999). Generally, the diagnosis of the *T. sergenti* infection is determined by detecting *T. sergenti* parasites in Giemsa-stained thin blood smear films by light microscopy. However, the diagnosis of *T. sergenti* is very difficult in asymptomatic or chronic infections since the parasitemia level is very low. In addition, the full life-cycle of *T. sergenti* remain obscure, as the intracellular parasites are very small and many of their morphological details are unrecognizable under the optical microscope (Hagiwara et al., 1997).

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However, the presence of parasitic DNA is evident throughout its life-cycle, and is not degraded by short-term environmental stress factors capable of altering transcriptional and post-transcriptional events. Therefore, molecular identification methods based on the genome of parasites are generally not limited to any particular development stage (Zarlenga et al., 2001). The introduction of the polymerase chain reaction (PCR) has allowed the development of specific and sensitive diagnostic methods for the detection of many types of piroplasmosis (Chae et al., 1996).

Some studies of *T. sergenti* infection in cattle based on microscopic examinations (Lee and Kim, 1987; Jeon, 1978) and PCR (Chae et al., 1996; Choi et al., 1997) have been previously reported in Korea.

This study was performed to investigate the prevalence of theileriosis and to compare the prevalence of this disease in Korean native cattle reared in two areas offering different environmental conditions, namely, a grazing area and a non-grazing area, by PCR.

MATERIALS AND METHODS

Cattle and blood sampling

Three hundred and one Korean native cattle (276 cows and 25 bulls) were examined for *T. sergenti* infection from 2001 to 2002. Two hundred and one grazing cattle in two large farms in the Seosan area (the Western area in Chungnam province) and 100 non-grazing cattle in five small farms in the Gongju area (Southern area of Chungnam province) participated in the present study. Blood samples were collected from the cattle when they were sampled for a routine health examination. None of the cattle had received treatment or a vaccination to block theileriosis.

The animal age range was from 10 months to 13 years old (mean 3.9 years old). All blood samples were collected from a jugular vein into anticoagulant EDTA bottle, and kept at 4°C for later use. Blood smears were prepared immediately, and parasitemia was examined by microscope.

DNA isolation

Two hundred microliters of whole blood was lysed in 0.1M Tris-HCl (pH 8.0) containing 1% SDS, 0.1 M NaCl and 10mM EDTA. The samples were then treated with proteinase K (100 µg/ml) for 2 hr at 55°C, and the DNA was extracted with phenol/chloroform, precipitated by ethanol, and dissolved in 50 µl of TE buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA). The DNA samples were then stored at 4°C for until required.

PCR amplification

The PCR primer set used was 5'-CCTCTTGAAGTCATCCATGT-3'(forward) and 5'-CACTGAGCTGGAAGAGCTA-3'(reverse), which are essentially the same as those used in a previous study (Chae et al., 2001). PCR amplification was performed in 50 µl of a mixture containing about 1 µg of template DNA, 1 µl each of the 20 pmol primers, 4 µl of 1.25mM dNTP, 1.0 U of Taq DNA polymerase (Gibco, USA), 5 µl of 10 × PCR buffer (100 mM Tris-HCl (pH 8.3), 500 mM KCl, 1.5 mM MgCl₂, and 0.1 % (w/v) gelatin). PCR amplification was initiated at 3 min at 96°C to activate the Taq DNA polymerase, and then over 35 cycles of denaturation for 30 sec at 96°C, annealing for 1 min at 60°C and extension for 1 min at 72°C. Five microliters of the PCR products obtained were analyzed by 2% agarose gel electrophoresis, which was followed by ethidium bromide staining and photography.

Specificity and sensitivity

Specificity and sensitivity of these primers was previously examined by Chae et al. (1996). Briefly, the expected 128 bp fragment obtained results from amplifying only the *T. sergenti* DNA and not bovine kidney cell DNA. *T. sergenti*-infected erythrocytes showing 0.3% parasitemia were subjected to two-fold serial dilutions with 0.9% normal saline, and the DNA was extracted from each diluted sample.

Statistical analysis

The data were analyzed with a database (SPSS v. 10.0, K). Statistical analysis of the prevalence was carried out with chi-square independence test.

RESULTS

T. sergenti infection in cattle

The parasitemia range of *T. sergenti*-positive cattle by microscopy ranged from 0.1 to 3% (mean 0.8%). All *T. sergenti* DNA-positive samples revealed a characteristic 128 bp band by 2% agarose gel electrophoresis (Fig. 1).

The prevalences of *T. sergenti* infection by PCR in

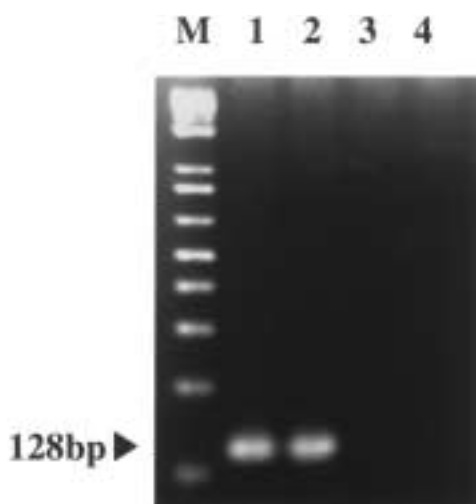


Fig. 1. Detection of *Theileria sergenti* DNA in Korean native cattle. The amplified 128-bp product was subjected to electrophoresis in 2% agarose gel and stained with ethidium bromide. M: 100-bp ladder, Lane 1: PCR-positive control of *T. sergenti*, Lane 2: *T. sergenti* DNA positive sample. Lane 3: PCR-negative control free of DNA template, Lane 4: *T. sergenti* DNA negative sample.

Korean native cattle are shown in Table 1. In terms of mean prevalence, 204 of the 301 Korean native cattle (67.8%) produced a positive PCR result. Our results also revealed that the infection rate of cows (70.3%) was significantly higher than that of bulls (40.0%) ($p < 0.01$), and that the over 3 year-old-group (75%) had a significant higher prevalence of *T. sergenti* infection than that less than 3 year-old-group (61.8%) ($p < 0.05$). Our data also showed that grazing areas (76.1%) had the significant higher prevalence than non-grazing areas (51%) ($p < 0.001$). Only 6 cattle of these 204 PCR positive cattle showed symptoms, such as anemia.

DISCUSSION

T. sergenti infection is associated with environmental factors such as, an intermediate host in cattle. Korea is located in a temperature zone, and is hot and humid during May to September, which is conducive for the growth and reproduction of ticks (Han, 1968). Han also reported that in Korean native cattle *T. sergenti* is a tick-transmitted infection, by mainly *Haemaphysalis longiconis*, which is most common during July.

Microscopic examinations have been used for the diagnosis of *T. sergenti* infection, because it is both inexpensive and straightforward, but many *T. sergenti*-infected cattle may not have been detected. On the other hand, PCR for *T. sergenti* infection in cattle using a pair of oligonucleotide primers, is a

Table 1. Prevalence of *T. sergenti* infection in Korean native cattle by PCR

	Non-grazing cattle (Gongju area)			Grazing cattle (Seosan area)			Mean		
	No. exam	No. pos	P. rate (%)	No. exam	No. pos	P. rate (%)	No. exam	No. pos	P. rate (%)
Sex									
Cows	75	41	54.7	201	149	74.1	276	194	70.3 ^{b)}
Bulls	25	10	40	0	0	0	25	10	40
Age (years)									
< 3	67	35	52.2	98	67	68.4	165	102	61.8
> 3	33	16	48.5	103	86	83.5 ^{a)}	136	102	75 ^{a)}
Total	100	51	51	201	153	76.1 ^{c)}	301	204	67.8

No. exam: number of examined, No. pos: number of positive, P. rate: positive rate.

^{a)}Significant statistical difference ($p < 0.05$).

^{b)}Significant statistical difference ($p < 0.01$).

^{c)}Significant statistical difference between two areas ($p < 0.001$).

highly specific and sensitive diagnostic tool. In the present study, we employed a pair of oligonucleotide primers, which target a 128 bp fragment in the ribosomal DNA of *T. sergenti*. These primers appear to be highly specific for *T. sergenti*, since no detectable amplification of *Babesia bovis* or bovine leucocyte DNAs was observed. In addition, the method was sensitive enough to detect parasite DNA in a 10 μ l blood sample with a calculated parasitemia of 0.00029% (Chae et al., 1996). Jeon et al. (1978) reported that the infection rate of *T. sergenti* by light microscopy was 68.9% in Korean native cattle.

In addition, Chae et al. (1996) reported that infection rates of *T. sergenti* in grazing Korean native cattle from two farms by microscopy and PCR method were 67.1% and 72.8%, respectively. Choi et al. (1997) also reported that the infection rate of *T. sergenti* in grazing Korean native cattle by microscopy and PCR were 64.8% and 88.7% in a big farm. The results revealed that positive reactions by microscopic examination and by PCR were 60.0% and 76.1% in grazing cattle (Seosan area), which is in agreement with the results of Chae et al. (1996) and Choi et al. (1997). In addition, *T. sergenti* infections in non-grazing cattle were 38.0% by microscopy and 51% by PCR in this study. The results also showed a slightly higher incidence in non-grazing cattle, but they were nevertheless lower than in grazing cattle.

Chae et al. (1996) reported that the parasitemia range in seven *T. sergenti* positive cattle by microscopy was from 0.1 to 6.2% (mean 1%). In our study, the parasitemia range of 158 *T. sergenti*-positive cattle by microscopy was from 0.1 to 3% (mean 0.8%), which are similar to the results of Chae et al. (1996). *T. sergenti* infection has been described as a benign theileriosis, even though infected animals sometimes develop severe clinical signs, like a high fever followed by anemia, weight loss and malaise (Rakha et al., 1999). In the present study, only 6 of the 204 PCR positive cattle showed symptoms like anemia, which demonstrates that many Korean native cattle might have benign theileriosis. Our results also reveal that the *T. sergenti* infection rate in the over 3 year-old-group (75%) was significantly higher than in the less

than 3 year-old-group (61.8%). These results indicate that *T. sergenti* is a persistent infection in Korean native cattle.

The infection rate of *T. sergenti* in cows (70.3%) was significantly higher than that in bulls (40.0%), however, further study is required in larger animal populations, as only 25 male cattle were examined in the present study. Animals in herds allowed more grazing days have a greater chance of being exposed to ticks, and consequently a greater chance of being infected with *T. sergenti* (Yamane et al., 2000). Our outcomes also show that the prevalence of *T. sergenti* infection in a grazing area was significantly higher than in a non-grazing area, which probably reflects the host's preferred environment (Lee, 1999).

In conclusion, this study demonstrates that the overall prevalence of *T. sergenti* infection is probably high in Korea, and that the infection is more common in grazing areas than in non-grazing areas. Therefore, life-long treatment and the development of an optimal vaccine for theileriosis are needed in both grazing situations.

REFERENCES

- Chae JS, Lee JM, Kwon OD, Park JH, Chae KS (1996) Rapid detection of *Theileria sergenti* by the polymerase chain reaction in Korean cattle. *Korean J Vet Res* **36**: 195-207 (in Korean).
- Chansiri K, Sarataphan N (2002) Molecular phylogenetic study on *Theileria* sp. (Thung Song) based on the thymidylate synthetase gene. *Parasitol Res* **88**: S33-35.
- Choi EJ, Kang SW, Kweon CH, Jeong WS, Yoon YD, Song HJ (1997) Rapid detection of *Theileria sergenti* by polymerase chain reaction. *Korean J Parasitol* **35**: 111-117 (in Korean).
- Hagiwara K, Takahashi K, Taniyama H, et al. (1977) Detection of *Theileria sergenti* schizonts in bovine lymph node. *Int J Parasitol* **27**: 1375-1378.
- Han TW (1968) Studies on the so-called small type piroplasma of cattle in Korea. II. studies on the ticks are transmitting so-called small type piroplasma. *Res Rep Rural Dev* **11**: 97-101 (in Korean).
- Jeon Y (1978) A survey on babesiosis and theileriasis in Korean cattle. *Korean J Vet Res* **18**: 23-26 (in Korean).
- Kim MC, Lee JM, Kwon OD, Chae JS, Kim HS (1993) A

- study for a construction of *Theileria sergenti* DNA probe. *Korean J Vet Res* **33**: 479-486 (in Korean).
- Kubota S, Sugimoto C, Onuma M (1996) Population dynamics of *Theileria sergenti* in persistently infected cattle and vector ticks analysed by polymerase chain reaction. *Parasitology* **112**: 437-442.
- Lee JG (1999) The textbook of veterinary parasitology. Korea textbook co. 2 nd ed. pp 361-364, Seoul, Korea (in Korean).
- Lee JM, Kim MC (1987) Studies for the effective diagnosis and treatment of bovine piroplasmiasis. *Korean J Vet Res* **27**: 321-330 (in Korean).
- Rakha NK, Sugimoto C, Onuma M (1999) Recent developments in diagnosis and prophylaxis of *Theileria annulata* and *T. sergenti* infections. *Curr Trends Immunol* **2**: 135-144.
- Savini G, Conte A, Semproni G, Scaramozzino P (1999) Tick-borne diseases in ruminants of Central and Southern Italy: epidemiology and case reports. *Parassitologia* **41**: S 95-100.
- Shiono H, Yagi Y, Thongnoon P, et al. (2001) Acquired methemoglobinemia in anemic cattle infected with *Theileria sergenti*. *Vet Parasitol* **102**: 45-51.
- Wang CT, Kubota S, Kakuda T, Kuo KK, Hsu TL, Onuma M (1998). Survey of *Theileria* parasite infection in cattle in Taiwan. *J Vet Med Sci* **60**: 253-255.
- Yamane I, Koiwai M, Tsusui T, Hamaoka T (2001) A survey of *Theileria sergenti* infection, daily weight gain and conception proportions in 85 herds of grazing heifers in Japan. *Vet Parasitol* **99**: 189-198.
- Zarlenga DS, Barry Chute M, Gasbarre LC, Boyd PC (2001) A multiplex PCR assay for differentiating economically important gastrointestinal nematodes of cattle. *Vet Parasitol* **97**: 199-209.