Genetic Diversity of Major Piroplasm Surface Protein Genes and Their Allelic Variants of *Theileria* Parasites in Thai Cattle

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**ABSTRACT.** Twenty-eight field isolated *Theileria* parasite DNAs obtained from dairy and beef cattle in distinct geographical areas of Thailand were characterized by using polymerase chain reaction (PCR) amplification with six sets of oligonucleotide primers. Three sets of them were modified from two genes of immunodominant major piroplasm surface protein (MPSP) coding for 32 kDa (p32) of *T. sergenti* and 33/34 kDa (p33/34) of *T. buffeli*, and MPSP of *Theileria* spp. (Thai-isolate). The other three sets of primers were basically generated from three alleles of MPSP which were specific for Japanese *T. sergenti*-Ikeda stock (I-type), Japanese *T. sergenti*-Chitose stock (C-type) and Australian *T. buffeli*-Warwick stock (B1-type), respectively. The results indicated that 14 out of 28 isolates were amplified by the Thai-specific primer whereas 6 isolates were amplified by the p32 specific primer and the other 5 isolates were amplified by the p32 and Thai-specific primers. In addition, by using the allele-specific PCR, 14 out of 28 isolates contained C-type MPSP whereas 3 isolates contained B1 type parasites. Interestingly, 20 out of 28 isolates could be amplified by the Thai-specific primer. The majority of *Theileria* parasites distributed in Thailand contained Thai type parasites, whereas C-type parasites showed the mixed population with B1 and Thai type parasites. No I type parasite was detected.—**KEY WORDS:** major piroplasm surface protein, PCR, isolates contained B1 type parasites. Interestingly, 20 out of 28 isolates could be amplified by the Thai-specific primer whereas 6 isolates were amplified by the p32 specific primer and the other 5 isolates were amplified by the p32 and Thai-specific primers. In addition, by using the allele-specific PCR, 14 out of 28 isolates contained C-type MPSP whereas 3 isolates contained B1 type parasites. Interestingly, 20 out of 28 isolates could be amplified by the Thai-specific primer. The majority of *Theileria* parasites distributed in Thailand contained Thai type parasites, whereas C-type parasites showed the mixed population with B1 and Thai type parasites. No I type parasite was detected.—**KEY WORDS:** major piroplasm surface protein, PCR, *Theileria*.—*J. Vet. Med. Sci.* 61(9): 991–994, 1999

The organism that causes theileriosis by infecting wild and domestic ruminants is a protozoan parasite belonging to the phylum Apicomplexa, family Theileriidae. Two species of *Theileria* parasites, *Theileria parva* and *Theileria annulata*, cause clinical disease in cattle commonly known as East Coast fever and Tropical theileriosis, respectively. Distribution of *T. parva* is limited in eastern, central and southern Africa, whereas *T. annulata* is more widely distributed in many tropical regions of the world, extending from southern Europe to southern Asia [2]. The other species of *Theileria* parasites cause mild disease in cattle well-known as benign bovine *Theileria* parasite, which are distributed in Asia, Europe and Australia. These groups are presumed to be *T. sergenti* [4, 16], *T. buffeli* [5] and *T. orientalis* [22].

The genes encoding for immunodominant major piroplasm surface proteins (MPSP) of 32 kDa (p32) of *T. sergenti* Chitose (C) stock, 33 kDa (p33) of *T. sergenti* Ikeda (I) stock and 34 kDa (p34) of *T. buffeli* Warwick stock had been reported [9, 10, 15, 21]. Recently, a genetic analysis MPSP allelic forms in mixed populations of *T. sergenti* and *T. buffeli* of Japanese cattle was studied by using allele-specific polymerase chain reaction (PCR) with 2 sets of oligonucleotide primers designed from nucleotide sequences of the p33/32 cDNAs of two Japanese stocks, I and C and one set of the p34 cDNA of *T. buffeli* (B1) Warwick stock [12, 13]. The results indicated that the majority of the stocks or field isolates are mixed population of parasites containing I and C-type MPSP alleles but imported cattle from Australia containing both C-type as well as B1-type MPSP allele. This study revealed that *T. sergentibuffeli* had been distributed in Japan. Furthermore, Kubota *et al.* [11] and Savini *et al.* [20] had also analyzed MPSP genes of *Theileria* parasite isolates from Australian and Italian cattle, respectively, using allele-specific PCR. The result showed that Australian benign *Theileria* parasites are composed of a combination with at least 2 types of B1- and C-type MPSP alleles and C-type MPSP allele is similar to that of *T. sergenti*. In 1971, the first report of *Theileria* parasites in Thai native cattle in the southern part of Thailand was observed by conventionally simple blood smear obtained from monitoring parasitaemia of the splenectomized calves. However, the species of the *Theileria* parasite was not notified [3].

In this study, we characterised field isolated *Theileria* parasite DNAs obtained from dairy and beef cattle in distinct geographical areas of Thailand in order to determine genetic diversity based on MPSP genes and their allelic types using PCR amplification.

**MATERIALS AND METHODS**

**Field isolates of Theileria parasites:** Twenty-eight field isolates of *Theileria* parasites used for the analysis were obtained from beef and dairy cattle with no clinical symptoms except enlarged prefemoral lymph nodes in some animals. All of the isolates had been diagnosed by microscopic examination of Giemsa-stained blood smear.

**Preparation of DNA and PCR:** Parasite DNAs were prepared for purified piroplasm according to previously
described by Sarataphan et al. [19] and finally treated with SDS-proteinase K solution and phenol extraction. Three sets of primers were used for PCR amplification of the parasite DNAs. The first set comprising of 5'-CAGCAGTTTGGCGAAGG-3' (Ts-u) and 5'-GGTGAGACTCAATGCGCCTA(Ts-R), respectively, to amplify the gene encoding for p32 of T. sergenti [21]. The second set was 5'-TATGTGTGCTGAGATCGT-3' and 5'-TGAGACTCAATGCGCCTAGA-3' oligonucleotides which amplified the genes encoding for p33/34 of MPSP genes as shown in Table 1. Group 1 contained 14 out of 28 isolates which were amplified by the Thai 3'-specific primer, indicating that the parasites of this group are Theileria spp. (Thai isolate). The result of allele specific MPSP revealed that the majority of parasites are Thai-type. However, 6 isolates in group 2 were only amplified by the p32-specific primer and 5 isolates in group 3 were amplified by both of the p32 and Thai 3'-specific primer. Group 4 consisted of 2 isolates which were both amplified by the p32- and p33/34- specific primers. These results indicated that there was a possibility of mixed population of T. sergenti and T. buffeli. Group 5 comprised of one isolate which was amplified by p32-, p33/34- and Thai 3'-specific primers.

Recently, Kakuda et al. [7] had analyzed the nucleotide sequences of MPSP genes derived from Theileria spp. (Thai isolate). A new oligonucleotide primer, Thai 3', was utilized as it is specific for Theileria spp. (Thai isolate). By using Ts-U and Thai3' primers for such purpose were basically derived from the conserved regions of the slow evolutionary genes among Theileria parasites, for example, small subunit ribosomal RNA [1] and house-keeping genes. The allelic form of MPSP may occurred as in the case of the MPSP of T. sergenti/buffeli/orientalis, T. parva, T. annulata and T. mutans [7].

Distribution of C, B1, and Thai-type MPSP parasites within field isolates in Thailand was relied on the data using allele-specific PCR as shown in Table 2. We found that 14 out of 28 isolates contained C-type MPSP, and 3 isolates were B1 type MPSP whereas 20 isolates were Thai-type. These results suggested that the majority (20/28) of Theileria parasites in Thailand contained Thai-type parasites. The minority (14/28) was C-type parasites which were found as a mixed population with Thai- and B1-type parasites. Similarly, the Australian Italian and Taiwan benign Theileria parasites are also composed of B1- and C-type [17, 23]. Nucleotide sequences of Australian C-type MPSP gene was similar to that of Japanese C-type parasite with 98.5% homology [11]. In addition, the data from Table 2 revealed that 14 isolates could not be amplified by using these 3 allele-specific primers. It is possible that the variation of nucleotide sequences within allelic form of various isolates of Theileria MPSP may occurred as in the case of the MPSP genes. Therefore, PCR detection of the Theileria parasites infection cannot merely rely on the use of either MPSP genes-specific or allele-specific primers. The suitable primers for such purpose were basically derived from the conserved regions of the slow evolutionary genes among Theileria parasites, for example, small subunit ribosomal RNA [1] and house-keeping genes.

The nucleotide sequences of cDNAs encoding p32 C and 1 type of T. sergenti were shown to be 85% homologous at amino acid level. Recently, Kubota and his colleagues [14] observed an alteration in parasite populations among I-type and C-type of T. sergenti during persistent infection in cattle and vector ticks. Parasite population changes were also apparent during persistent infection in cattle over several
months, and this change leads to the suppression of host immune response [14]. This suggested that the co-existence of multiple allelic variants of the major surface molecule in an infected host may disturb host defense mechanisms. Monoclonal antibodies against p32 derived from C and I type parasites showed that the antibodies recognized only homologous parasites but not heterologous ones indicating that I and C-type MPSP are antigenically different [6].

According to the data in this study, it can be summarized that field isolates of *Theileria* parasites which closely related to *T. sergenti* and *T. buffeli* are distributed in Thailand. The majority of *Theileria* parasites contained Thai-type parasites, whereas minority of C-type showed the mixed population with Thai- and B1-type parasites.

REFERENCES


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Table 1. Number of field isolates of *Theileria* parasites from beef and dairy cattle in Thailand classified into 5 groups based on MPSP genes

<table>
<thead>
<tr>
<th>Group No. of Isolates</th>
<th>Breeds&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Location&lt;sup&gt;b&lt;/sup&gt;</th>
<th>MPSP genes</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>p32</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
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<tr>
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<td>NT</td>
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<td>3</td>
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</tr>
<tr>
<td>5</td>
<td>1</td>
<td>HF</td>
<td>2</td>
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<sup>a</sup>HF: Holstien Freissan, AB: American Brahman, NT: Native cattle, AFSS: Australian Freisian Sahewal.

<sup>b</sup>1=Thung Song, Nakhon Si Thammarat, 2=Rattapum, Songkla, 3=Tab Kwang, Saraburi, 4=Ban Pong, Ratchaburi.

<sup>c</sup>Thai: Theileria buffeli are distributed in Thailand. The minority of *Theileria* parasites showed that the antibodies recognized only homologous parasites but not heterologous ones indicating that I and C-type MPSP are antigenically different [6].

According to the data in this study, it can be summarized that field isolates of *Theileria* parasites which closely related to *T. sergenti* and *T. buffeli* are distributed in Thailand. The majority of *Theileria* parasites contained Thai-type parasites, whereas minority of C-type showed the mixed population with Thai- and B1-type parasites.

Table 2. Number of *Theileria* parasite isolates from beef and dairy cattle in Thailand based on type of MPSP alleles

<table>
<thead>
<tr>
<th>No. of isolates</th>
<th>C</th>
<th>I</th>
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<th>Thai</th>
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<tr>
<td>14</td>
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