Survey of *Theileria* Parasite Infection in Cattle in Taiwan

Chun-Tsuen WANG, Shuichi KUBOTA 1), Tsutomu KAKUDA 1), Chih-Chi KUO, Tien-Lai HSU 2) and Misao ONUMA 1)

Veterinary Hospital, College of Agriculture, National Taiwan University, Taipei, Taiwan, R. O. C., 1)Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060, Japan and 2)Council of Agriculture, Taipei, Taiwan, R. O. C.

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ABSTRACT.  An survey of *Theileria* parasite infection in cattle in Taiwan was carried out by polymerase chain reaction (PCR). A total of 491 blood samples, 105 from southern area and 386 from northern area, were collected from bovine in 16 different farms. From northern area, *Theileria* piroplasms could be seen in only 4 of 105 blood samples microscopically. However, when p32/34 genes (encoding immunodominant piroplasm surface proteins) were amplified by PCR, 15 blood samples were detected positive. They were analyzed by using allele-specific primers of 3 allelic forms of p32/34 and all contained C type of *T. sergenti*. Four blood samples were found infected with both C and B (*T. buffeli*) type parasites. Examination of 386 blood samples from southern area of Taiwan did not reveal any *Theileria* parasite microscopically, as well as by PCR amplification. — KEY WORDS: PCR, Taiwan cattle, *Theileria sergenti.*

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Bovine piroplasmosis due to *T. sergenti* is a major cause of economic loss in grazing cattle in Japan. The cDNA coding a parasite-specific protein can be used as a diagnostic means of detecting protozoan parasites. Previously, we developed the polymerase chain reaction (PCR) technique to specifically amplify a gene of a major piroplasm surface protein, p32 of *T. sergenti*, and it is significantly more sensitive than the serological test [7]. Among *T. sergenti* isolated from different areas in Japan, genetic diversities were detected by Southern blotting using probes of genomic DNA clones [9] and cDNA clones encoding a p32 [8]. Most parasite stocks and field isolates consisted of mixed populations [12] of genetically and antigenically different parasites.

To differentiate parasite population bearing 3 allelic forms of p32/34 of *T. sergenti/buffeli*, 3 sets of oligonucleotide primers were designed to amplify each of 3 alleles by PCR [6, 7]. By using this allele-specific PCR, we found that the majority of *T. sergenti*-infected calves in Japan harbored mixed parasite populations bearing Ikeda (I) and Chitose (C) types of *T. sergenti*. In contrast, *T. buffeli* (B) was detected in cattle imported from Australia to Japan [5, 6]. Benign *Theileria* spp. are widespread among cattle in eastern and south Asian countries.

The herd of cattle in Taiwan was infected with piroplasm as an agent of tick fever [4]. Chang [1] and Su et al. [10] substantially reported that *Theileria* parasites from grazing cattle in Taiwan were similar to *T. sergenti* morphologically. In this report we describe the survey of *T. sergenti/buffeli* infection in cattle in Taiwan and analysis of field isolates by allele-specific PCR.

Blood samples were collected from northern and southern parts of Taiwan. In northern Taiwan, 105 bovine blood samples were collected from 5 dairy farms (Holstein breed), one Livestock Research Institute experimental farm and one yellow cattle farm; whereas in south, 386 bovine blood samples were also collected from 7 dairy farms (Holstein breed) and 2 Livestock Research Institutes. The dairy cattle herds were housed in stall throughout the year. The beef cattle and dairy cattle raised in 3 Livestock Research Institutes were maintained in enclosures or in pastures. Yellow cattle were raised in pasture. Giemsa-stained blood smears were examined under a microscope.

Parasite DNA was prepared from parasitized bovine erythrocytes as described previously [8]. The oligonucleotide primers (20–25 mers) for PCR, Ts-universal (Ts-U) and Ts-reverse (Ts-R) primers are described elsewhere [11]. For allele-specific PCR, three sets of oligonucleotide primers were designed to amplify each of three alleles as shown in Fig. 1, and used in combination.

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**Fig. 1.** Schematic representation of the genes coding for the p32 of *Theileria sergenti* piroplasm. The positions of the oligonucleotide primers used for PCR amplification are indicated by arrows.
with Ts-R.

For the reaction, 50 µl of a mixture containing 5–50 ng of DNA as a template, primers (1 µM each), deoxynucleotide triphosphate (200 µM each) and 1.25 U of Taq polymerase (GIBCO BRL Life Technologies Inc., U.S.A.) in 1 x PCR buffer (20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2.0 mM MgCl₂) were used. The reactions proceeded in a programmed temperature control system (Model PC-700 ASTEC Co., Ltd., Fukuoka, Japan) for 35 cycles. Each cycle consisted of 1 min of denaturation at 94°C (4 min for the first cycle), 1 min of annealing at 73°C and 1 min of polymerization at 72°C, with an additional 3 min at 72°C after the last cycle. After amplification, 5–10 µl of each sample were subjected to agarose gel electrophoresis. Southern blotting of PCR product was carried out as described previously [8] using ³²P-labelled cDNA clone L9–1 probe derived from C type parasite of T. sergenti [8].

Detection of p32/34 genes of T. sergenti/buffeli in blood samples from cattle in Taiwan were carried out by PCR method described above. The results are shown in Table 1. Positive cases (15 out of 30) were found in only one farm (SH Livestock Research Institute, Northern Taiwan). The blood samples from the other farms in both northern and southern areas were negative by PCR. Of 30 blood smears from the SH LRI, examined microscopically, only 4 smears contained Theileria parasite in a few erythrocytes (Table 2).

All of the 15 amplified PCR products reacted with a probe (C type of T. sergenti) by Southern blot analysis (Fig. 2). To determine the allelic form of p32/34 of Theileria parasite, allele-specific PCR was performed using 3 sets of the specific primers. As shown in Table 2, all of the 15 samples contained C type but not I type of T. sergenti, and only four samples contained mixed populations of T. sergenti C type and T. buffeli (B type). B type parasites are further subdivided into B-1 (T. buffeli type) and B-2 (found in Japanese isolates but not in Australian isolates) by restriction enzyme pattern of the amplified bands [2, 6], and 4 Taiwan isolates with B type were all B-1 type.

Several papers described the isolation and serological diagnosis of Theileria parasite infection in Taiwan in the past [1, 4, 10]. However since the last report in 1985 [10], there has been no article about survey of Theileria parasite infection in Taiwan. In the present experiment, we conducted the survey of Theileria parasite infection by using the more sensitive diagnostic method, PCR. Of 491 blood samples collected from Taiwan, 15 (about 3%) were positive and all belonged to one farm (Northern area) where T. sergenti infection has been reported previously [1, 10].

Majority of T. sergenti-infected calves in Japan presented mixed parasite populations bearing I and C type parasites [7]. T. buffeli is distributed mainly in Australia and in adjacent areas in Asia [3, 5]. By using 3 sets of primers (for I, C, and B type), we attempted the typing of Taiwan Theileria isolates. All of the isolates contained C type parasites and 4 samples showed mixed populations of C and B type parasites. In this study, I type parasite could not be identified from Taiwan, while in Japan and Korea, I type is the major parasite distributed in the field [2, 5]. There is no report concerning the relation between the allelic form and virulence of T. sergenti/buffeli. However, there are several suggestive evidences that I type is more pathogenic than C and B type parasites; and Ikeda (I type) stock is more pathogenic than Fukushima (C type) stock. In Korea, all Theileria isolates contained I type parasite and showed severe clinical symptoms [2], whereas in Taiwan, all isolates contained C type parasites but not I type parasite and showed no clinical symptoms. T. buffeli is known to be benign parasite. The relationship between allelic forms and virulence of T. sergenti is now under investigation.

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REFERENCES


Fig. 2. PCR amplification of *T. sergenti* DNA in cattle from SH LRI and Southern blot analysis of amplified products. Right lane shows the PCR amplification and left lane shows the Southern hybridization.