Comparison of Partial Ribosomal DNA Sequences of Babesia gibsoni Occurring in Miyazaki Prefecture, Japan

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ABSTRACT. Nucleotide sequences of ribosomal DNA (rDNA) of Babesia (B.) gibsoni occurring in Miyazaki, western Japan, were examined using blood samples obtained from seven dogs suffering from natural canine babesiosis. DNA isolated from these blood samples was subjected to the polymerase chain reaction (PCR). The nucleotide sequences of the PCR products were determined and compared with other rDNA sequences of B. gibsoni isolated from Asia, Europe and U.S.A. Although homology values between our isolates and those isolated from Europe and U.S.A. were both 84.0\%, respectively, our isolates were identical to the Asian types. In conclusion, B. gibsoni occurring in Miyazaki was revealed to have the genotype Asia 1 or Asia 2 from a comparison of the partial rDNA sequences.

KEY WORDS: Babesia gibsoni, canine babesiosis, ribosomal DNA.

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Babesia (B.) canis and B. gibsoni are known to cause canine babesiosis [5, 8, 10]. Babesiosis caused by B. gibsoni occurs in Africa and Asia, but rarely in the U.S.A. and Europe. In Japan, canine babesiosis is caused by B. gibsoni. Among several DNA-based methods for detection of Babesia infection, polymerase chain reaction (PCR) has been shown to be useful [3]. Nucleotide sequences of ribosomal RNA gene (rDNA) amplified by PCR can be used for examining phylogenetic relationships among Babesia strains [13]. The rDNA sequences of Babesia species provide useful information about their genetic relationships [1, 4, 6, 7, 9, 11–13]. In the present study, we determined the partial sequences of the rDNA and compared them with other reported sequences of B. gibsoni to investigate the phylogenetic position of B. gibsoni occurring in Miyazaki prefecture, western Japan.

Blood samples infected with B. gibsoni were collected from seven naturally infected dogs in Miyazaki prefecture. For the PCR experiment, DNA was extracted from whole blood obtained from the infected dogs. DNA extraction and PCR were carried out as described previously [2]. Briefly, DNA was extracted from 200 μl of infected blood using phenol-chloroform. The primers PIRO-F (5’-AGTCATATTGCTAATTGTAGG-3’) and PIRO2-R (5’-TGT-TATTTCTTGACTACCC-3’), which produce 309-bp products. The PCR products were electrophoresed on 2.0% SeaKem ME agarose (FMC, Maine, U.S.A.) gel, and stained with ethidium bromide. A band of the PCR product was excised from the gel and purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The purified PCR products were subjected to direct sequencing in an ABI 377 automatic DNA sequencer (Perkin-Elmer, Foster City, CA, U.S.A.).

The nucleotide sequences of the PCR products originating from Miyazaki (“Miyazaki strain”) were examined for their homologies with the nucleotide sequences of B. gibsoni strains isolated in Europe and U.S.A., and with B. gibsoni genotypes Asia 1 and Asia 2 obtained from GenBank (accession number: B. gibsoni 18S rDNA, AF188001; B. gibsoni 16S rDNA, L13729; B. gibsoni genotype 1, AF175300; B. g. g. genotype 2, AF175301), using a computer software program (GENETYX-WIN Version 3.2.0, Software Development Co., Ltd., Tokyo). All the sequences of “Miyazaki strain” were identical to each other. Comparisons of the nucleotide sequences between “Miyazaki strain” and other B. gibsoni rDNAs are shown in Fig. 1. The nucleotide sequence of “Miyazaki strain” and those of B. gibsoni isolated in Europe and U.S.A. were not closely related, and the homology values were both 84.0\%, respectively. However, our isolates were almost identical to Asia 1 and Asia 2 of B. gibsoni.

The small subunit ribosomal DNA gene is considered to be an appropriate gene for phylogenetic analysis because it shows only limited variation in its nucleotide sequence [4]. Therefore, it was examined in some Babesia species [1, 4, 6, 7, 9, 11–13]. B. gibsoni strains isolated from Asia, Europe and U.S.A. were compared with each other [12, 13]. B. g. g. isolated in U.S.A. by Thomford et al. (unpublished
The data was closely related with 18S rDNA of *B. gibsoni* isolated at Europe by Zahler et al. (90.4% similarity) [12] and not closely with the Miyazaki strain (84.0%). Zahler et al. reported two other different sequences of 18S rDNA isolated from dogs in Japan, Malaysia and Sri Lanka [12, 13]. These two sequences from Asia 1 and Asia 2 showing a close relationship (99.9% similarity). However, they were not closely related to *B. gibsoni* rDNA isolated in U.S.A. and Europe (88.0–89.6% similarity) [12, 13]. The results of our homology analysis suggest that the PCR products of “Miyazaki strain” might be closely related to Asian type of *B. gibsoni*, but different from *B. gibsoni* isolated in Europe or U.S.A. The present results might help to clarify the phylogenetic position of *B. gibsoni* occurring in Miyazaki prefecture, although our PCR products represent only part of the rDNA. For further studies, we may need to obtain a complete sequence of *B. gibsoni* rDNA so as to compare it with various samples isolated from other parts of the world.

REFERENCES