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Abstract. We demonstrate here the identification and phylogenetic characterization of *Babesia microti* (*B. microti*)-like parasite detected from a splenectomized Japanese macaque (*Macaca fuscata fuscata*) at a facility for laboratory animal science. On Day 133 after splenectomy, intra-erythrocytic parasites were found on light microscopic examination, and the level of parasitemia reached 0.3% on blood smear. Molecular characterization of the parasite using nested-polymerization chain reactions targeting the 18S rRNA, β-tubulin, and subunit 7 (eta) of the chaperonin-containing t-complex polypeptide 1 (*CCT7*) genes were identified as a *B. microti*-like parasite, designated the Japanese Macaque *Babesia*-1 (JM-1).

The genus *Babesia* belongs to the family Piroplasmida, closely related to *Plasmodium* and *Theileria* genera, and comprises over 70 species that parasitize mammals and birds. *Babesia microti* (*B. microti*) is a rodent-infective *Babesia* species transmitted by ixodid ticks and is also a major etiologic agent of human babesiosis. Nonhuman primates in Africa and Asia are natural hosts for *Entopoploides macaci*, of which is a piroplasm phylogenetically close to the *B. microti* parasite and similar in morphology. *B. microti* parasites have recently been reported to infect various vertebrate hosts such as the raccoon, domestic dog, fox, squirrel, sus and cynomolgus macaques, is an Old World monkey species native to Japan. It is found only in Japan and classified in controlled Biosafety Level II condition at Tsukuba Primate Research Center, given commercial food pellets supplemented with fresh fruits, and maintained in accordance with the Guidelines for the Use of Experimental Animals authorized by the Japanese Association for Laboratory Animal Science. The protocol was approved by the Ethics Committee of Animal Experiments, Dokkyo University of School of Medicine (permit no.: 0536).

Although no marked clinical signs were observed in the monkey during the postoperative period after splenectomy, at 133 days after operation intra-erythrocytic parasites were found on light microscopic examination. Parasitemia reached 0.3% spontaneously, and dot forms (Figure 1A), ovoid forms measuring about 2 μm in diameter (Figure 1B), pyriforms (Figure 1C), and ring-forms (data not shown) were frequently detected on Giemsa-stained thin blood smears. In addition, multiply-infected erythrocytes were often observed (Figure 1D). The parasites were morphologically distinct from primate malaria parasites, but were very similar to *B. microti*.

Polymerase chain reaction (PCR) was performed using genomic DNA (gDNA) of the parasite from the peripheral blood. Heparinized blood obtained from J79 was centrifuged at 1,200 × g for 10 min at 4°C. Erythrocytes were washed three times with phosphate-buffered saline (PBS) by centrifugation at 1,200 × g for 10 min at 4°C, and the buffy coat was removed completely. The gDNA was extracted using a QIAamp DNA blood mini kit (Qiagen, Tokyo, Japan) according to the manufacturer’s instructions. The gDNA was used as a template for nested PCR, which was carried out targeting the 18S rRNA, β-tubulin, and subunit 7 (eta) of the chaperonin-containing t-complex polypeptide 1 (*CCT7*) genes of *Babesia* and *Theileria*, as described previously with minor modifications.

The 18S rRNA gene was amplified using *Piro*0F/Piro6R for the first-round PCR and *Piro*1F/Piro 5.5R for nested PCR (Table 1). The β-tubulin gene was amplified using the primers TUB-ATG5F/Tubu-1538R for the first-round PCR and Tubu-63F/Tubu-3R for nested PCR (Table 1). The *CCT7* gene was amplified using the primers TBCCCT35F/TBCCTR0 for the first round, and TBCCCT70F/TBCCCT1519R-3 for nested PCR (Table 1). The PCR reaction

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The Inc., Brea, CA) with the DTCS DNA Sequence kit (Beckman
Coulter, using a CEQ8000 automated sequencer (Beckman
Coulter, Vista, CA). Nucleotide sequences of
mixture contained 0.1 μg of template DNA, 5 μL of 10× PCR
buffer with 15 mM MgCl2, (TaKaRa Bio Inc., Shiga, Japan),
5 μL of dNTP mix (2 mM of each dNTP) (TaKaRa), 2.5 U of
Takara LA Taq DNA polymerase (TaKaRa), and 50 pmol of
each primer set for the 18S rRNA, β-tubulin, or CCT7 gene-
specific primers for PCR as described previously with minor
modifications as reported.21

Nestedy PCR successfully amplified 18S rRNA, β-tubulin,
and CCT7 genes from the gDNA of J79 (data not shown). The
PCR products were isolated by 1% (w/v) agarose-gel elec-
trophoresis in TAE buffer and purified with a GENECLEAN
kit (BIO 101, Inc., Vista, CA). Nucleotide sequences of 18S
rRNA, β-tubulin, and CCT7 genes were then determined
using a CEQ8000 automated sequencer (Beckman Coulter,
Inc., Brea, CA) with the DTCS DNA Sequence kit (Beckman
Coulter). The 18S rRNA, β-tubulin, and CCT7 genes were
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TABLE 1

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Primers</th>
<th>Oligonucleotide sequences (5’ to 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18S rRNA</td>
<td>Piro 0F</td>
<td>GCCAGTAGTGCATATGCTTGTGTATA</td>
</tr>
<tr>
<td></td>
<td>Piro 6R</td>
<td>CTCCTTCTCTTAAGTGAATAAGGTACCAC</td>
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<tr>
<td></td>
<td>Piro 1F</td>
<td>CATCGCATGTCTWGTAYAAPTTTTTA</td>
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<td></td>
<td>Piro 5.5R</td>
<td>CTTYTAAGTGAATAAGGTACCAAACCT</td>
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<td></td>
<td>TUBL-ATG5F</td>
<td>ATGGAGARATYGTACATAYCAAGC</td>
</tr>
<tr>
<td></td>
<td>Tubu-1538R</td>
<td>TAYTGTGTGTAYTCGTCRACYA</td>
</tr>
<tr>
<td></td>
<td>Tubu-63F</td>
<td>CAAATWGGYGCMAARTTYTGGGA</td>
</tr>
<tr>
<td></td>
<td>Tubu-3R</td>
<td>TCAGTCCATACTCTTCCSGTTRACCGT</td>
</tr>
<tr>
<td>β-tubulin</td>
<td>TBCCT35F</td>
<td>TGAAGGARGGNACNGAYACWTCYCAARGG</td>
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<td></td>
<td>TBCTC70F</td>
<td>GTYWTCRTCDATDSWNAGNACHWGCGANGCGYCTCDGTNGC</td>
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<tr>
<td></td>
<td>TBCTC1519R-3</td>
<td>KTYYTYTNTACMANBHDGYTCCCADATRCA</td>
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</table>
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