Detection of *Theileria annulata* carrier cattle by PCR

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A simple method for treating bovine blood samples for direct detection of *T. annulata* in carriers, after polymerase chain reaction (PCR) amplification of small subunit ribosomal RNA (SSU rRNA) gene is described. The threshold of detection of the PCR-assay was an erythrocytic parasitaemia of 0.00008% corresponding to 16 infected bovine erythrocytes. In 50 known carriers, 42 were positive in PCR, in which 8 cattle revealed presence of *T. annulata* in stained blood smear under microscope.

Cattle recovered from acute infection with *Theileria annulata* remain persistently infected with low, microscopically undetectable levels of the organisms. Little information is available about the role played by these carrier animals in transmission of the disease because of inability for detection and quantitation of the low level infection. The method of detection involving serological tests are not suitable for this purpose. In this report, a PCR based assay capable of detecting 0.00008% erythrocytic parasitaemia equivalent to 16 piroplasms of *Theileria annulata* has been described.

The piroplasms of *T. annulata* were separated from blood of infected bovine calf at the height of erythrocytic parasitaemia (35%) by holding the tubes at 95°C for 10 min. Piroplasm DNA (10 ng) and each of the treated blood samples (5 μl) were subjected to PCR amplification in 100 μl reaction mixture. The mixture contained 40 p. mol of each of the primers (Table 1), 200 μM of each of the four dNTPs, 1.5 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.1% Triton X-100 and 3 U Taq DNA polymerase (Genei, Bangalore). Each PCR cycle consisted of a denaturing step (97°C, 1 min), an annealing step (60°C, 2 min) and an extension step (72°C, 3 min). The PCR was performed for 29 cycles of amplification (PTC-200 DNA engine, MJ Research, USA). The amplified products were detected by electrophoresis on 1.2% agarose gel mixed with ethidium bromide.

SSU rRNA gene primers amplified 372 bp fragment of the genomic DNA of *T. annulata*. The result demonstrated that the lowest detection limit of the PCR-assay was an erythrocytic parasitaemia of 0.00008% equivalent to 16 bovine erythrocytes infected with piroplasms (Fig. 1). No specific amplification of 372 bp DNA fragment was recorded in blood samples of cattle infected with other coenzootic haemoproteozoa, namely *Babesia bigemina* and *Trypanosoma evansi*. PCR-assay was more sensitive than the parasite detection by light microscopy as 42 of 50 (84%) known carrier of

<table>
<thead>
<tr>
<th>Primer (No.)</th>
<th>Sequence 5’-3’</th>
<th>Specificity</th>
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<tbody>
<tr>
<td>989</td>
<td>AGTTTTCTGACCTATCAG</td>
<td><em>Theileria</em></td>
</tr>
<tr>
<td>1347</td>
<td>TGCAACAGACCCAGAGG</td>
<td><em>T. annulata</em></td>
</tr>
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Fig. 1—Threshold of detection of *T. annulata* in blood by PCR (Lanes 1 to 5: blood samples with 0.02, 0.05, 0.001, 0.0003, 0.00008% parasitaemia respectively, 6: no DNA 7: piroplasm DNA and 8: molecular size marker).

*T. annulata* were detected positive by PCR as compared to the microscopical detection of the parasite in only 8 (16%) animals. Earlier, a PCR-assay using oligonucleotide gene primers encoding the 30 kDa major merozoite surface antigen of *T. annulata*, detected 75% positivity in 92 carrier cattle in which only 22% showed microscopically detectable parasites in blood smear.

References