

Thesis Title	Development of the Detection of <i>Babesia bovis</i> in Cattle by Polymerase Chain Reaction
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Abstract

Babesia bovis is a tick-transmitted protozoan parasite causing babesiosis in cattle. Because of its lethal pathogenicity to cattle, endemicity of babesiosis affects livestock industry in Thailand. Thus, development of procedures for detection the parasite is essential for appropriate therapeutic purposes and epidemiology studies.

A specific DNA probe for *B. bovis* detection, pMU-B1, has already been cloned. The 6.0 kb insert was subcloned into Bluescribe M13(-) vector at *Sau3AI* site. Sixteen clones which gave positive signals when probed with labeled *B.bovis* DNA were obtained from colony hybridization. pMU-BS7 was selected as a candidate because of its highest signal per insert size. pMU-BS7 contained 329 bp with 49.5 % G+C content. The amplification primers were designed from 329 bp fragment for the semi-nested PCR in order to enhance the specificity and sensitivity of *B.bovis* detection. The amplification product of outer (BV2 and BV3) and inner pairs (BV1 and BV2) were 182 bp and 155 bp, respectively. The assay was specific for *B.bovis* since no amplification was detected with *Babesia bigemina*, *Trypanosoma evansi*, *Anaplasma marginale*, *Theileria sp.*, or bovine leukocyte DNA. Using this semi-nested PCR method, the limit of sensitivity

of detection of *B.bovis*-infected blood in ethidium bromide stained agarose gel was 0.0002% parasitemia, and using DNA hybridization was down to 0.00002 % parasitemia, which is equivalent to 10 parasites in 10 μ l blood. In an experimentally infected cow, parasites were detected by the semi-nested PCR method (ethidium bromide stained agarose gel) at days 8 post-infection, 2 days earlier than microscope examination, and could not be detected 3 days after treatment of the cow with Berenil (3 mg/Kg body weight).