Thesis Title Development of specific DNA probe to detect *Babesia bovis* infection in cattle.

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Abstract

Babesia bovis is a tick-transmitted, intraerythrocytic protozoan parasite causing babesiosis in cattle. Because of its lethal pathogenicity to cattle, endermicity of babesiosis affects the development of livestock industry. Thus, it is essential to develop improved identification procedure for diagnosis and epidemiological studies.

A genomic library of *B. bovis* of a Mexican strain (Kb) has been constructed in a plasmid pUN 121 and cloned in *E. coli*. Several clones which hybridized strongly to radioactively labeled *B. bovis* genomic DNA in an *in situ* screening have been selected and studied. It was found that pMU-B1 could detect 25 pg of *B. bovis* DNA which was equivalent to 1000 parasites in a 10 µl volume of whole infected blood, or 0.001% parasitemia. No detectable cross-hybridization was observed with *Babesia bigemina*, *Trypanosoma evansi*, *Plasmodium falciparum*, *Anaplasma*

marginale, tick, and cow DNAs. The pMU-B1 contains 6.0 kb of *B. bovis* insert which has been fine-mapped by various restriction enzymes. The insert was found to contain AccI, AvaI, KpnI, NdeI, and SpeI sites. It was also found that the DNA insert was present at high copy number and it appeared to be distributed throughout the parasite genome. The pMU-B1 could also differentiate a Mexican (Kb) strain from Thai (TS#4, TS#4a) strains. Thus, the pMU-B1 probe will be useful in the diagnosis of *Babesia* infection in cattle and tick, differentiation of strains, and determination of virulence with a particular strain of *Babesia*.

For the used of this DNA probe in field studies, the detection by non-radioactive probes has been preliminary investigated. By digoxigenin-deoxyuridine triphosphate (dig-dUTP) labeling, enhanced chemiluminescence (ECL), and biotin labeling methods, the probes were found to detect at least 50, 2000, and 5000 pg of purified *B. bovis* DNA, respectively.