

Thesis Title DNA Sequence Analysis of a Specific *Trypanosoma evansi* DNA Probe and Its Use for a Highly Sensitive Detection of *T.evansi* in Blood.

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Date of Graduation 30 April B.E. 2537 (1994)

Abstract

Trypanosoma evansi is a haemoflagellated parasitic protozoa which is mechanically transmitted by blood sucking insects, especially by Tabanid flies. It causes a syndrome referred to as "surra", an emaciating and sometimes fatal disease, targeting a wide range of livestock. Since *T.evansi* poses an important problem for the animal health and production development program, especially in the endemic areas inside and outside Thailand, a practical method for a highly sensitive and reliable detection is needed. To this end, a method for direct detection of the parasite's DNA by polymerase chain reaction (PCR) was developed.

A previously developed *T.evansi* genomic DNA probe, pMUTec6, was subcloned into Bluescribe M13⁻. The clone pMUTec6.258 was selected for its signal intensity after hybridization with the *T.evansi* genome and for its size, suitable for PCR amplification. pMUTec6.258 showed an overall 50% GC content in its 258 bp sequence. From this sequence, a set of primers for PCR was designed allowing amplification of a 227 bp fragment from the original sequence. The amplification conditions were optimized for high sensitivity down to the detection of a single parasite

in 10 μ l blood. In an experimentally infected cow, the parasites were detected as early as 2 days post-infection and the signal totally disappeared 12 hr after successful treatment of the cow. The 227 bp amplification was specific for the detection of trypanosomes of the brucei group, *T.brucei* and *T.equiperdum*. No amplification product was seen from host blood in bovine, swine, equine, ovine, and caprine species, or from the following other blood parasites: *Anaplasma* sp., *Theileria* sp., *Babesia bovis*, and *Babesia bigemina* DNA.

For easier manipulation during the sample-collections, the blood processing method was simplified by boiling the crude blood for at least 5 min prior the amplification. Either clotted- or EDTA anticoagulated blood, both from tube-collected and slide-collected could be used as starting materials for the developed PCR method. Seventy-one field specimens were tested for evaluation, the sensitivity and specificity of the PCR detection when compared with the mouse inoculation method was 100% and 88% respectively.