Thesis Title Development of DNA probes for sensitive detection and isolates differentation of Trypanosoma evansi

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ABSTRACT

Trypanosoma evansi is known to be parasitic protozoa that causes disease in livestocks called "Surra". T. evansi infection causes emaciation in animals . Due to Surra that plays an important role in animal health, methods for parasite detection should be developed. The classical medthods of microscopic observation of parasite in blood stream, mouse inoculation and serological assays are not reliable enough to carry on in intensive animal industry of today . DNA probe hybridization method is therefore developed for T. evansi identification and characterisation.

EcoRI genomic DNA library of T.evansi Npl isolate was screened for repetitive DNA fragment that specific for only T.evansi. From 575 recombinants there was pTec.6, of 6.5 kb insert in pUN121 vector which performed the most strong positive nucleic acid hybridization. As little as 62.5 pg DNA or 10^3 parasites was detectable by Dot-blot hybridization with pTec.6 and no cross hybridization was found in Cow DNA, Buffaloe DNA and other two blood parasites DNA.

kDNA was also constructed to be sensitive probe candidates. It was found to have variation in term of copy number in individual isolates.

No method that can charaterize T. evansi for its subspecies. The strain differentiation probe was constructed. Sua3AI NpII isolate DNA library was screened for specific [1A fragment. pTec.21 of 122 bp insert exhibited subspecies specific DNA fragment that gave Restriction Fragment Length Polymorphisms (RFLPs) in Southern hybridization of HhaI digested DNA. The pattern was distinguishable among isolates. So far, there were ten different RFLP patterns from 14 isolates.