Form No.5

Abstract of Dissertation

Applicant

Doctoral Program in Animal and Food Hygiene

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Title : Development and validation of immunochromatographic test (ICT) for diagnosis of animal trypanosomosis

(家畜トリパノソーマ病診断用イムノクロマトグラフィー法の開発と評価に関する研究)

Abstract:

Trypanosome infection is the worldwide distributed disease. Various species of animal including water buffaloes, horses and cattle have been affected leading to huge economic losses dues to reduction of the animal products (milk, meat, fur etc...). Moreover, endemic areas of the disease are often located in the countryside where diagnostic laboratories are costly or inaccessible. Therefore, effective and accurate field test is of great interest to the scientists and so the farmers. My study aimed to develop and validate an immunochromatographic test (ICT), a rapid, sensitive and simple method for detection of animal trypanosomosis. For that purpose, identification of a novel antigen was an important and foremost step.

Chapter 1 describes the production of tandem repeat (TR) antigen TeGM6-4r as a novel diagnostic antigen for animal trypanosomosis. The newly expressed TeGM6-4r demonstrated higher immune-reactivity to water buffaloes' sera, which had been experimentally infected with *T. evansi,* compared to the previously characterized TbbGM6-2r, and clearly distinguished positive samples from negatives in ELISA. TbbGM6-2r and TeGM6-4r are identical in amino acid sequence, but different only in the number of repetitiveness. In ELISA, OD value of TR antigen increases with increasing number of the repeat unit. This was explained by the increase of the epitope located in the repeat unit

which provides more binding sites for the antibodies. Another advantage of TeGM6-4r was that since amino acid sequence of GM6 is highly conserved among the salivarian trypanosome, using a single universal antigen could be able to detect several *Trypanosoma* spp. including *T. evansi*, *T. congolense* and *T. vivax*.

Chapter 2 describes the evaluation of the TR antigen TeGM6-4r using ELISA. The antigen was 100% specific to *Theileria* and *Babesia spp.*, and 81.4% to *T. theileri*. TeGM6-4r based ELISA demonstrated 100% specificity and 80% sensitivity in detection of *T. evansi* infection in experimentally infected water buffaloes. In the field condition, TeGM6-4r/ELISA had a sensitivity of 86.3% and specificity of 58.3% comparing to CATT/*T.evansi*, The test detected higher number of positive sera than CATT. This might be attributed to cross-reaction with *T. theileri*; however, existence of *T. theileri* among water buffaloes in Vietnam still needs to be confirmed. In utilizing TeGM6-4r for surveillance of surra among water buffalo in Northern Vietnam, it was found that the disease was still widely endemic in the region. Seroprevalence was 27% detected by CATT/*T. evansi*. Anti-TeGM6-4r antibodies were detected in 53% of the animals. The high seroprevalence illustrates the widespread occurrence of *T. evansi* in Northern Vietnam at the present. This result indicated that TeGM6-4r-based ELISA might be an effective and useful tool for epidemiological survey of surra in water buffaloes.

In Chapter 3, the on-side diagnostic test TeGM6-4r/ICT was constructed subsequently. The test demonstrated remarkable performance. In detection of *T. evansi* infection from experimentally infected water buffaloes, TeGM6-4r/ICT was comparable to TeGM6-4r/ELISA which could clearly distinguish 100% negative and positive controls. The test was able to detect *T. brucei*, *T. congolense* and *T. vivax* in field derived samples obtained from Ugandan and Tanzanian cattle. The result was in agreement with parasitological test and showed substantial agreement with *T. b. brucei* and *T. congolense* lysate antigen/ELISAs and TeGM6-4r/ELISA (kappa value 0.64, 0.72 and 0.78 respectively). This was the first time an ICT is developed and evaluated for detection of animal trypanosomosis. The test utilized TR antigen TeGM6-4r and the recombinant DNA technology, therefore was able to be produced with high quality and large quantities. Although sensitivity of TeGM6-4r/ICT was relatively lower than TeGM6-4r/ELISA, it could detect both IgG and IgM in the serum samples which more advantageous than ELISA. While CATT is only available for *T. evansi* detection, ICT is

able to recognize *T. evansi*, *T. congolense* and *T. vivax*.

In Chapter 4, the TeGM6-4r based ELISA and ICT was validated for diagnosis of animal trypanosomosis among sheep, goats and cattle in Kwazulu-Natal province, South Africa. This was the first time a variety of serodiagnostic tests has been evaluated for diagnosis of trypanosome infections in South Africa. Both ELISA assays utilizing crude and recombinant (TeGM6-4r) trypanosome antigens were highly sensitive and efficient. The TeGM6-4r ICT was less sensitive than ELISA however is relatively specific, simple and rapid. Remarkably, the ICT results were highly in agreement with the results obtained from PCR method, which means ICT was able to detect the truly active infections. Seroprevalence of animal trypanosome infection was variably detected in different serodiagnostic tests applied in this study. The prevalence in ovine, caprine and bovines was 0 - 44.4%; 0 - 9.1%, and 19.9 - 29.0% respectively. There were no positively detected ovine samples by CATT/*T. evansi*, whilst none of the caprine samples were positively detected by ICT. Similar to previous animal trypanosomoses prevalence reports in South Africa, the disease is more prevalent in cattle. Considering the high number of samples collected in this study, goats appear to be less susceptible to animal trypanosome infections or rather less preferable host for the trypanosome vector as compared to cattle. Goats are considered as important reservoirs for trypanosomes, therefore they should be taken into consideration in all programs aimed at controlling the disease.

In conclusion, an ICT utilizing a novel antigen TeGM6-4r were successfully developed. The antigen demonstrated high sensitivity (86.3%) and specificity (58.3-100%) in detection of *T. evansi* infection among water buffaloes in Northern Vietnam. The TeGM6-4r/ICT was able to detect both *T. congolense* and *T. vivax* infections in cattle, sheep and goats in Uganda, Tanzania and South Africa. The test was comparable to parasitological test, PCR and TeGM6-4r/ELISA. With further improvement on sensitivity the trypanosome ICT has the potential for use in both research and on-site diagnosis in trypanosome endemic countries. Beside animal trypanosomosis detection the test may also be a candidate for detection of human African trypanosomosis.

Notes 1. Fill in the Japanese translation for an English in the ( ).

2. Abstract should be between 1,800 and 2,200 characters in Japanese, or be between 1,000 and 1,400 words in English.