Form No.5

Abstract of Thesis/Dissertation

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Title：Studies on the development of dourine specific diagnostic methods

(媾疫特異的診断技術の確立に向けた研究)

Abstract

Livestock industry is a major economic division in many developing countries. Mongolian livestock sector contributes 90% of the total agricultural production, which accounts for 11% of total GDP in Mongolia. Horse population is over 2 million heads, and horse are considered as the most important domestic animal with high economic value due to their central role in nomadic pastoralism as well as source of meat and dairy product in Mongolia. Non-tsetse transmitted horse trypanosomosis (dourine and surra), which are important infectious protozoan diseases, are distributed in many countries including Mongolia. Dourine a lethal animal trypanosomosis in equidae which is caused by *Trypanosoma equiperdum* infection. Many trypanosome are transmitted by blood sucking insects, while, *T. equiperdum* is transmitted from infected horse to healthy horse via coitus. Because of this unique infectious strategy of *T. equiperdum*, the disease can easily transmit by a stallion to all of mares in herd. Therefore, dourine is a disease of great economic importance for horse production sector. Effective diagnostic methods for dourine to control the disease is highly expected, however, none of the effective and field- friendly diagnostic methods are developed until now. Additionally, there are no practical diagnosis markers for distinguishing between dourine and surra due to the lack of genome information of *T. equiperdum*. The diagnosis marker based on species specific

nucleotide sequences and/or genes may be found by the whole genome comparison between *T. evansi* and *T. equiperdum*.

For these reasons, in this study, I aimed that evaluation of *T. evansi* GM6 (rTeGM6-4r)-based diagnositic methods (ELISA and immunochromatographic test (ICT)) for the sero-diagnosis and sero-epidemiological study against dourine in chapter 1. Furthermore, in order to develop novel diagnosis tools based on species-specific nucleotide sequences of *T. equiperdum* in future, the construction of draft genome of *T. equiperdum* IVM-t1 strain and comparative genomics were performed in chapter 2.

In chapter 1, I evaluated the potential diagnostic abilities of rTeGM6-4r based diagnostic methods for dourine using Mongolian horse samples. This recombinant protein has already shown good diagnostic value in surra in other livestock species. First, genetic diversities of GM6 antigen were determined by sequencing among subgenus *Trypanozoon*. Ninety-seven percent amino acid sequence homology of GM6 was found among subgenus *Trypanozoon*. Moreover, anti-rTeGM6-4r antibody universally recognized GM6 antigens in *Trypanozoon* trypanosomes in indirect fluorescence antibody test (IFAT). These findings suggested that the utilities of rTeGM6-4r based sero-diagnostic methods for both surra and dourine. Next, I examined 50 blood samples collected from a trypanosomosis outbreak-suspected horse farm in Mongolia. In addition to the blood samples, genital organ swabs were collected from three selected horses with clinical signs of dourine. The diagnostic values of an rTeGM6-4r based ELISA or ICT were measured in comparison to the result of a *T. evansi* crude antigen based ELISA as a reference test, which is recommended by OIE terrestrial manual. The positive serum samples were detected in 46%, 42%, and 28% of the tested horses, using the rTeGM6-4r based ELISA, crude antigen-based ELISA, and rTeGM6-4r based ICT, respectively. Moreover, the sensitivity and specificity of rTeGM6-based ELISA were 81% and 79%, respectively. The kappa value between reference test and rTeGM6-based ELISA was 0.6, which was considered as moderate. While, the sensitivity and specificity of rTeGM6-4r based ICT were 57% and 93%, respectively. The kappa value between reference test and rTeGM6-4r based ICT was 0.53. Additionally, the motile trypanosomes were found by microscopic observation and the trypanosome specific band was shown by PCR using the samples collected from genital organs with clinical symptoms. On the other hand, none of the PCR positive sample were found using DNA extracted from whole blood. These results highly indicated that the horses were infected in dourine, but not infected in surra. In general, the rTeGM6-4r based ELISA and ICT represented as useful diagnostic options for non-tsetse transmitted horse trypanosomosis, especially for dourine.

In chapter 2, I focused on sequencing and processing of whole genome DNA of newly isolated *T. equiperdum* IVM-t1 strain. First, total genomic DNA was extracted from culture adapted *T. equiperdum* IVM-t1 strain and sequenced by two different types of next generation sequencers, MiSeq and PacBio. Genomic sequence data, which generated from MiSeq, had a high degree of accuracy of nucleotide sequencing although it had shorter reads. On the other hand, PacBio generated a longer sequence reads but less accuracy of nucleotide sequencing. By assembling the raw data from two next generation sequencers by multiple softwares, I finally constructed the draft genome of *T. equiperdum* IVM-t1. Assembly quality and contig numbers of the draft genome of *T. equiperdum* IVM-t1 were significantly improved in comparison with previously published the genome of *T. equiperdum* OVI. This improved *T. equiperdum* genome data has long been needed for further studies on *T. equiperdum* and dourine. Additionally, chromosome construction, gene prediction of *T. equiperdum* IVM-t1 were performed using the published genome of *T. brucei* TERU927 as reference by Companion pipeline. As a result of comparative genomics analyses between *T. equiperdum* IVM-t1 and *T. brucei* TERU927, 11 chromosomes and around 8,000 gene candidates including around 600 *T. equiperdum* IVM-t1-specific genes were predicted. Moreover, these results suggest that species-specific genes were related to species-specific parasitism. Comparative genomics among trypanosomes is strongly expected for discovery of species-specific genes and identification of their function in further studies. At this stage of the study, whole genome draft assembly and intrinsic gene prediction produced by the current study provides a resource for future trypanosome genetic studies and has revealed some *T. equiperdum* specific genes. The phylogenic analysis based on the amino acid sequence of 50 core proteins revealed the close relationship among subgenus *Trypanozoon*. Furthermore, these results can be extended by transcriptome based more accurate gene annotation and more sophisticated genetic analysis using this available resource. Species-specific diagnostic methods will be developed based on these data resource of whole genome sequencing.

The two chapters indicated that rTeGM6-4r based ELISA and ICT can be applied in sero-epidemiological study and in diagnosis of dourine in horse. And the whole genome data of *T. equiperdum* IVM-t1 must be an important research resource for the development of new diagnostic method for dourine in the future.

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3. Do not include figures and tables.

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